Antiopella cristata (Delle Chiaje, 1841) - Italy, Ionian Sea: Santa Maria La Scala

Antiopella cristata (Delle Chiaje, 1841) (Nudibranchia Janolidae). This nudibranch is distributed in the Mediterranean Sea and along the European Atlantic coasts as far as Norway. *Antiopella cristata* may reach 75-80 mm in length and presents an oval shape body, slightly flattened dorso-ventrally. The body may be transparent white or orange. Rhinophores are united at the base and between them there is a crest, called caruncle. Cerata are numerous, located latero-dorsally and form lateral series on either side united around the frontal margin in front of the rhinophores. They are finger-like, swollen and smooth. Through the transparent cerata, tributaries of the digestive gland can be observed. The tip of the cerata is light blue transparent and white spotted. On the notum, usually there are two white parallels lines that run throughout the body, from the head to the tail. The egg masses have a wavy pearl ribbon shape and may be pink-colored or white-colored. Antiopella cristata feeds on erect bryozoans as *Bugulina turbinata* (Alder, 1857), *Bugula* spp., *Bicellariella ciliata* (Linnaeus, 1758), *Alcyonidium* spp., *Bugularia* spp. and *Cellaria* spp. This species lives in shallow and in sheltered areas, on rocky bottoms where bryozoans, which it feeds on, live. In the central-eastern coasts of Sicily, A. cristata specimens are not very common and have been found from 11 to 36 m of depth. The majority of *A. cristata*'s specimens were observed on the following substrates: red algae with detritus, a mix of red and brown algae, photophilous assemblages, red algae, *Bugula* spp. and *Crambe crambe* (Schmidt, 1862). The egg masses were laid on *Bugula* spp., on *Dictyota* spp., on the central branch of *Eudendrium* sp. covered by bryozoan and on *Carpodesmia zosteroides* (Turner) Greville. *Antiopella cristata* seems to be an annual species that starts to appear in autumn, reaching the peak of its presence in winter and spring and tend to disappear at the beginning of summer.

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Biodiversity Journal

A new species of genus *Psalidodon* Eigenmann, 1911 related to the *P. paranae* complex (Characiformes Characidae) from Upper Paranaíba river basin, Brazil, supported by genetic and morphometric data

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ABSTRACT   The genus *Psalidodon* Eigenmann, 1911 (Characiformes Characidae) is a fish group with great diversity, expressed at the chromosomal, genetic and taxonomic level. The genus is marked by events of allopatric and vicariant evolution, by the formation of complexes of species and by wide geographical distribution. Both in these fish and other organisms, the association of studies with molecular markers and geometric morphometric techniques are useful in delimiting significantly evolutionary units (ESU). In this work, we performed maximum likelihood estimates (MLE) from mitochondrial Cyt b gene sequences and canonical variables (CVA) from 13 landmarks in eight populations of *P.* aff. *paranae* Eigenmann, 1914. The analysis of Maximum likelihood resulted in the structuring of populations in two different clades, one of which was composed only of individuals from a small population inhabiting a stream with approximately two km of length, demonstrating their clear distinction from the other populations. The analysis of canonical variation demonstrated the complete structuring of this population, and the position of each clade in the morpho-space was congruent with the topography observed in the MLE. Based on the results found, the existence of a new endemic species of the genus *Psalidodon* is evident.

KEY WORDS   Endemism; geometric morphometry; mtDNA; vicariance.

INTRODUCTION

The genus *Psalidodon* Eigenmann, 1911 (Characiformes Characidae) is part of the former genus *Astyanax* S.F. Baird et Girard, 1854, that is widespread in the Neotropicalis widespread of Neotropical ichthyofauna, with model species in developmental and evolutionary studies (Jeffery, 2001, 2008; Borowski, 2009). Such former genus contains at last 170 species (Eschmeyer et al., 2020), meanwhile *Psalidodon* 38 species so far, inhabiting almost all Brazilian rivers and constituting a significant fraction of the fish studied at the chromosomal and genetic level (Pasa & Kavalco, 2007).

Recently, Terán et al. (2020) moved species of

Astyanax to other six genera. More specifically, while the species complexes *A. fasciatus* (Cuvier, 1819) and *A. scabripinnis* Jenyns, 1842 were relocated to *Psalidodon*, and the *Astyanax* species from the coastal river basins of Brazil, like *A. giton* Eigenmann, 1908, were relocated to *Deuterodon* Eigenmann, 1907, the ones from the complex *A. bimaculatus* (Linnaeus, 1758) and the North American species remain in the *Astyanax* genus.

Psalidodon paranae (Eigenmann, 1914) is a small fish species inhabiting headwaters of rivers and streams from the Upper Parana River basin, belonging to a larger species complex group, formerly subspecies of *P. scabripinnis* (Eigenmann, 1927).

Moreira-Filho & Bertollo (1991) proposed that *P. scabripinnis* should be a group of cryptic species, based on cytogenetic and morphometric characters in populations of the upper Paraná and São Francisco river basins, where they are widely distributed (Bertaco & Lucena, 2006). However, one can even consider *P. paranae* as a group of cryptic species that occur in the Upper Paraná River Basin, because of different diploid number and several distinct karyomorphs (2n = 50, 2n = 48 and 2n = 46) (see Pasa & Kavalco, 2007 for a review).

Despite morphometric and chromosomal features, several studies in genus *Psalidodon* and other cryptic fish species used mitochondrial sequences to elucidate their distinctiveness. Rocha et al. (2019) used morphometrics and cytochrome b (cyt b) sequence to demonstrate the distinctiveness between *P. paranae* and *P. rivularis* Lütken, 1875, both formerly subspecies of *P. scabripinnis* from Upper Parana and São Francisco river basins, respectively. Kumar et al. (2017) also used cytB to show three mitochondrial lineages with high genetic variation and haplotypic diversity in *Ompok bimaculatus* Bloch, 1974. Using the same tool, Zhu et al. (2016) found moderate to high levels of genetic differentiation in 11 populations of *Scomber japonicus* Houttuyn, 1782, with two of them well structured, showing high diversity besides the other nine with low ones.

In this way, we aim to demonstrate a new*Psalidodon* species based on morphometric and Cyt b mitochondrial sequences. We sampled this species only isolated in a small stream used for water supply, endangered by an anthropic disturbance menace.

MATERIAL AND METHODS

We sampled eight populations of *Psalidodon* aff. *paranae* from Upper Paraná river hydrographic system, Paranaíba river Basin (Fig. 1, Table 1). After sampling, we brought the living specimens to the laboratory, euthanized them according to the technical standards of CONCEA - National Council for Control of Animal Experimentation of Brazil and CEUA/UFV - Animal Use Ethics Committee/Federal University of Viçosa (760/2018). We performed the sampling with licenses provided by SISBIO - Biodiversity Authorization and Information System (1938128) and SISGEN - National System for the Management of Genetic Heritage and Associated Traditional Knowledge (A9FE946). After that, we deposited the samples under vouchers numbers in the Ichthyology Collection of the Federal University of Viçosa, Campus Rio Paranaíba (supplement). We checked their species identities according to their morphological diagnostics (Oliveira, 2017).

We extracted the total genomic DNA from liver and heart samples according to the manufacturer instructions (Invitrogen PureLink DNA extraction and purification kit). We used the primers H16460 (CGAYCTTCGGATTACAAGAC) and GluDG.L (TGACCTGAARAACCAYCGTT) (Perdices et al., 2002) to amplify the cytochrome oxidase b gene by PCR (according to Prioli et al., 2002) and sequenced the samples with a private company (Myleus, Belo Horizonte, MG, Brazil). Sequences was deposited on GenBank, accession number MK756216 to MK756259.

We visualize the obtained sequences, align them with ClustalW v1.6 (Thompson et al., 1994) and calculate the genetic distances in MEGA X (Chernomor et al., 2016; Kumar et al., 2018), with *P. rivularis* specimens as outgroup. To reconstruct the Maximum Likelihood phylogram, we used the software MEGA X (Kumar et al., 2018), with the substitution model HKY (Hasegawa et al., 1985) according to the test of models in the same software.

We photographed every sampled individual with a Sony Cybershot camera (14.1 megapixels), and we prepare the files with TPSUtil 1.64 (Rohlf, 2013). We delimited thirteen anatomic landmarks with TPSDig2 2.32 (Rohlf, 2015) with 1 cm of scale factor to include the objects in an X/Y coor-
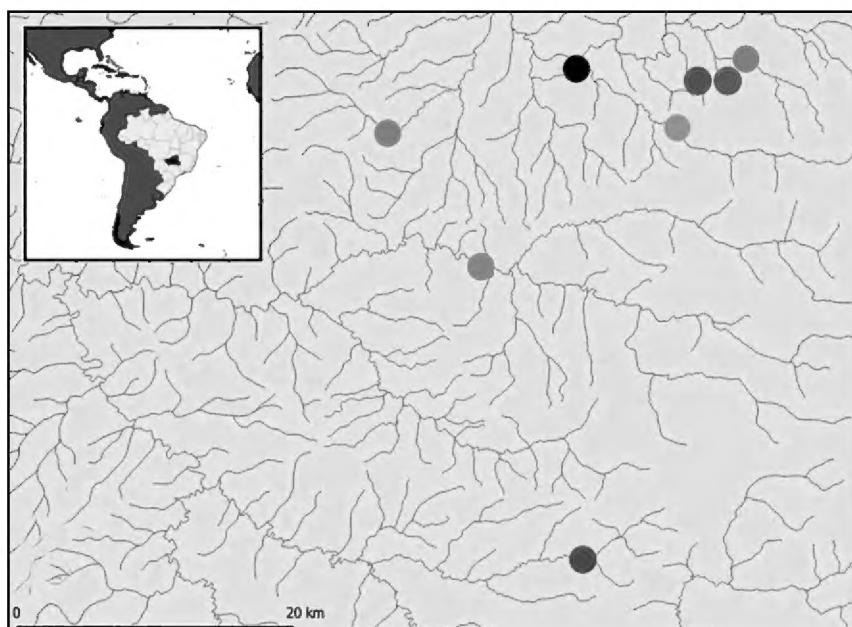
Figure 1. Distribution of samples in the Paranaíba river basin: Água Grande stream (red); Rita stream (light blue); Lava-pés stream (green); Olhos d'Água stream (gray); Paranaíba river (black); São João river (pink); Fora river (orange) and Quilombo river (dark blue).

Collection points	Geographical coordinates	Morphometry	*Cyt b*
Água Grande stream	19.177967"S 46.22613"W	9	3
Lava-Pés stream	19.191615"S 46.257387"W	10	3
São João river	19.308256"S 46.397117"W	8	6
Paranaíba river	19.182286"S 46.332794"W	10	10
Rita stream	19.187714"S 46.236178"W	7	6
Fora river	19.2232"S 46.45889"W	10	3
Quilombo river	19.49413"S 46.33163"W	10	3
Olhos d'Água stream	19.21285"S 46.27091"W	11	3

Table 1. Collection points, geographical coordinates and number of individuals used in each analysis.

dinates system (Figs. 2, 3). In MorphoJ 1.06 (Klingenberg, 2011), we made the Generalized Procrustes Analysis (GPA) in order to remove the size, rotation and orientation effects from the sample. We also searched for outliers and checked the normal distribution. We used two criteria as classifiers: collect point and clade (clade 1 = Lava-pés stream, Olhos d'Água stream, Fora river, Paranaíba river and Água Grande stream; clade 2 = Quilombo river and São João river; and clade 3 = Rita stream; according to ML phylogram). We verified the homoscedasticity of data with Procrustes ANOVA.

To minimize the effects of allometry on the sample, we performed a multivariate regression of the Procrustes coordinates vs centroid size. For a discriminatory analysis between populations and clades, we carried out the canonical variation analysis (CVA), and included it in both analyzes a permutation test for null hypothesis for the average of equal groups, using 10,000 permutation rounds.

RESULTS AND DISCUSSION

All individuals were identified *a priori* as *P. paranae* according to description available. We did not found any diagnostic characters distinguishing any of populations.

The Maximum Likelihood shows a structuring of the 38 individuals into two principal clades, excluding the outgroup, *P. rivularis* (Fig. 4). One of them is composed only with individuals from Rita stream, indicating a clear genetic differentiation between this population and the others. Such differentiation is corroborated by the genetic distance between population, meanwhile a low genetic distance is present within (Table 2).

The values of the Procrustes ANOVA test rejected the null hypothesis both in the analysis between populations (SS = 0.0393, MS = 0.0003, df = 154, F = 3.87, p <0.0001) and between clades (SS = 0.0181, MS = 0.0004 , df = 44, F = 5.59, p <0.0001).

In the analysis of canonical variation, it was possible to observe that the population from the Rita stream was structured along CV2 (responsible for 24.9% of the variation), whereas in CV1 (responsible for 30.9% of the variation), it was possible to observe a tendency to structure the populations of Água Grande stream and Fora river (Fig. 4). The permutation test rejected the null hypothesis (p <0.05) among all populations, with the exception of Olhos d'Água stream and São João river, and Paranaíba and São João rivers.

In the analysis of canonical variation between clades, it was possible to observe a clear structuring of clade 3 (Parque das Exposições stream) in relation to clades 1 and 2 along CV1, responsible for 66.2% of the variation. Clades 1 and 2 showed a tendency to structure themselves along CV2, responsible for 33.8% of the variation (Fig. 5). The permutation test rejected the null hypothesis (p <0.01) among all clades.

Through the Wireframes corresponding to the

ends of each axis of the CVA responsible for structuring the Rita stream or Clade 3 (Figs. 5, 6), it was possible to observe that the main morphological difference between this population and the others is the shorter caudal peduncle.

Using morphometric and genetic information, we demonstrate that the *Psalidodon* population from the Rita stream represents, in reality, a new species, inhabiting a stream approximately two km in length, surrounded by populations of *P. paranae*, which we describe below.

Psalidodon rioparanaibanus n. sp. (Fig. 3)
http://zoobank.org/urn:lsid:zoobank.org:104718CE -F240-4832-89F2-76A9DB879E83

EXAMINED MATERIAL. Holotype (LaGEEvo 4011 - L12), Rita stream, Rio Paranaíba, Minas Gerais, Brazil, 19.187714"S 46.236178"W, 21 Aug 2017, I.B. da Silva & M.A. da Silva. Paratypes (LaGEEvo 4236, 4277, 4279, 4280, 4281, XXX1, XXX2 - L13), da Rita stream, Rio Paranaíba, Minas Gerais, Brazil, 19.187714"S 46.236178"W, 23 Aug 2018, I.H.R. Oliveira, I.B. da Silva, G.K. Leles, R.L. Oliveira, T. Castanho.

DESCRIPTION OF HOLOTYPE. A vertically elongated humeral spot, the body more robust in the anterior part, short snout smaller than the diameter of the orbit, darker black sideband at the base of the caudal peduncle and close to the humeral spot, complete lateral line, dorsal fin with 11 rays being nine-branched, caudal fin with 17 rays being 14 branched, eight rows of scales above the lateral line and six below, 33 scales perforated along the lateral line.

VARIABILITY. Dorsal fin with 10–11 rays with 8–9 branched, anal fin with 16–19 rays with 14–18 branched, 7–9 rows of scales above the lateral line and 4–6 below, 33–36 perforated scales along the sideline.

ETYMOLOGY. Specific name in allusion to the municipality of Rio Paranaíba where the type series was discovered.

DISTRIBUTION AND BIOLOGY. *Psalidodon rioparanaibanus* n. sp. is endemic to the da Rita stream, a tributary of the Paranaíba river, belonging to the Upper Paraná River basin.

REMARKS. Conserved environments have more stable environmental characteristics, which favours the establishment, adaptation and development of a population (Frankham et al., 2002). This pattern can occur in the case of *P. rioparanaibanus* n. sp., which has a clear distinction from the *P. paranae* analyzed populations and represents a significantly evolutionary unit (ESU). The location of this point is in a green area within the city of Rio Paranaíba, which does not suffer constant human interventions that hinder the conservation of the place since the water supply of Rio Paranaíba municipality depends on this stream. Also, the stream is isolated from other by a small waterfall (about 2 meters high), followed by a long steep slope through a pasture. The isolation of the population meant that the
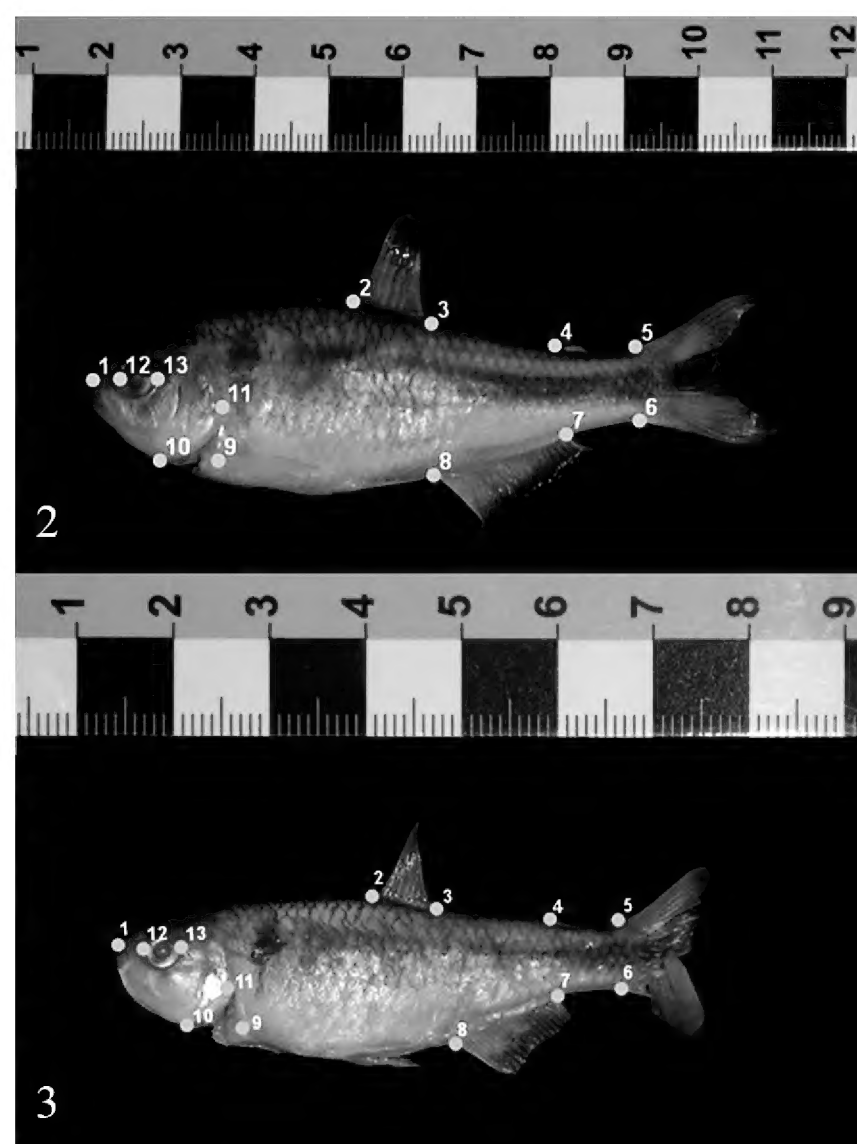
Figure 2. *Psalidodon paranae* specimen demonstrating the landmarks used in the analysis of geometric morphometry: 1 - snout tip; 2 - anterior insertion of the dorsal fin; 3 - posterior insertion of the dorsal fin; 4 - anterior insertion of the adipose fin; 5 - insertion of the first ray in the dorsal margin of the caudal fin; 6 - insertion of the first ray in the ventral margin of the caudal fin; 7 - posterior insertion of the anal fin; 8 - anterior insertion of the anal fin; 9 - insertion of the pectoral fin; 10 - ventral limit of the opercular opening; 11 - end of the opercular curve; 12 - anterior margin of the orbit and 13 - posterior margin of the orbit. Figure 3. *Psalidodon rioparanaibanus* n. sp.

genetic differences accumulated over time were more evident when compared to the other populations analyzed in this work (Table 2).

Along with genetic divergence, *P. rioparanaibanus* n. sp. differed from *P. paranae* in all morphometric analysis (Figs. 5, 6), with the p-value associated with discriminatory tests following the observed distinction of this population on the score charts. Geographic isolation can contribute not only to genetic divergence but also to morphology since

some of the fastest responses presented by populations to environmental pressures are related to changes in the morphology of individuals (Streelman & Danley, 2003).

Small and "closed" populations, such as that of the Rita stream, tend to face severe problems with loss of genetic diversity and increased inbreeding (Table 2). These factors together reduce the ability to respond to changes in the environment and adapt, making it difficult for a population to remain in a
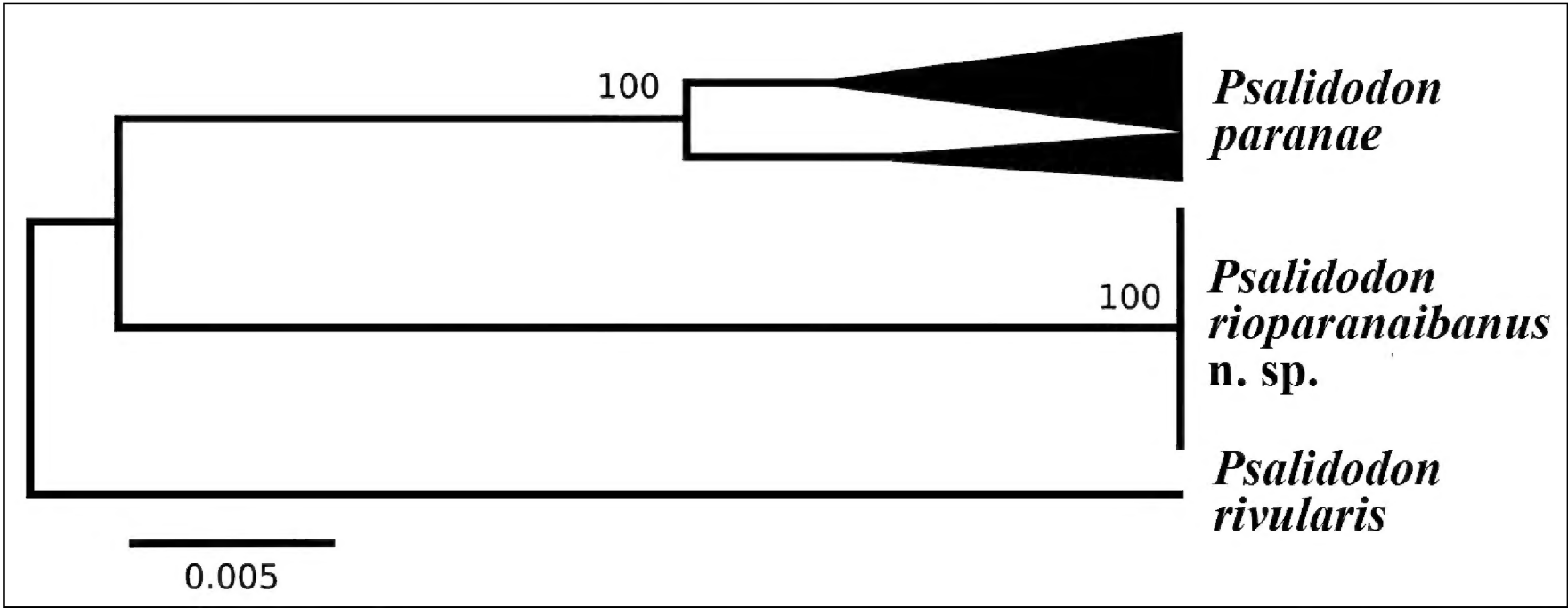
Figure 4. Phylogram resulting by (ML) Maximum Likelihood from the cyt b mitochondrial gene sequences of the specimens of *Psalidodon paranae*, *P. rioparanaibanus* n. sp. and *P. rivularis* as outgroup. The evolutionary history was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model. The tree shown above is the one with the highest log likelihood (-1141.58). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 38 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 653 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

	1	2	3	4	5	6	7	8
1 – Olhos d'Água stream	**0.000**							
2 – Quilombo river	0.011	**0.000**						
3 – São João river	0.013	0.000	**0.001**					
4 – Rita stream	0.048	0.044	0.048	**0.000**				
5 – Lava-pés stream	0.002	0.010	0.011	0.046	**0.002**			
6 – Água Grande stream	0.004	0.012	0.013	0.049	0.003	**0.003**		
7 – Paranaiba river	0.003	0.011	0.011	0.048	0.001	0.002	**0.001**	
9 – Fora river	0.002	0.011	0.012	0.049	0.002	0.003	0.001	**0.001**

Table 2. Genetic distance between *Psalidodon paranae* populations. Diagonally and in bold, distance within populations.
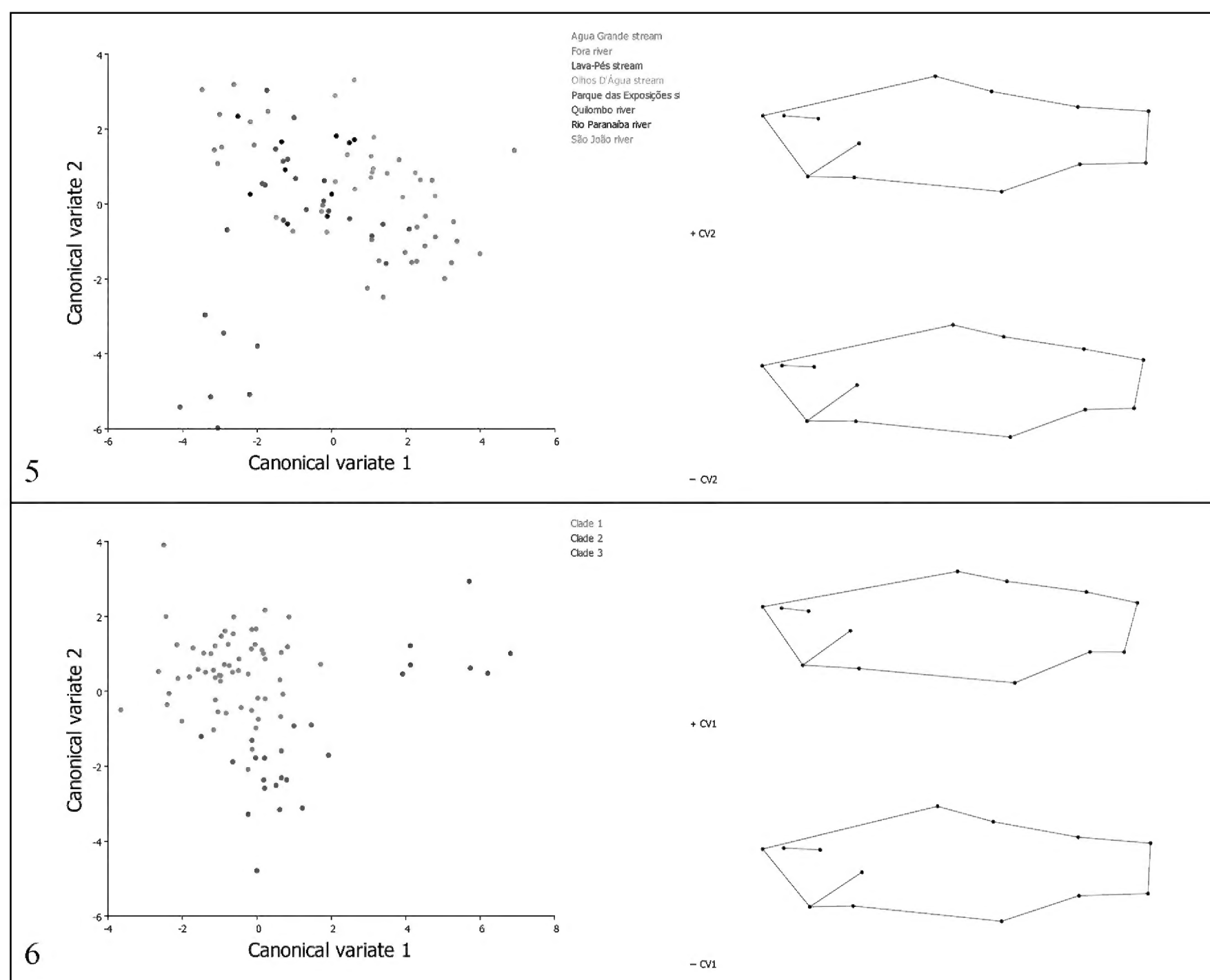
Figure 5. Morpho-space of CVA between collection points and Wireframes containing the shape found at the ends of CV2, responsible for structuring the Rita stream. Figure 6. Morpho-space of CVA between clades and Wireframes containing the shape found at the ends of CV1, responsible for structuring clade 3 (Rita stream) related to clades 1 and 2 (other populations).

given location (Hedrick & Kalinowski, 2000). Besides, genetic homogeneity between individuals caused by the high rate of inbreeding can lead to the elimination or drastic reduction of the population in the face of stochastic events that can happen in the environment, such as deforestation and reduction of the area for the permanence and adaptation of individuals. In small populations, the effects of genetic drift are also more significant, causing even greater impacts on their evolution (Frankham et al., 2002).

Along with this, there are issues related to habitat fragmentation and the consequently reducing migration, which directly influence the reduction of gene flow and increase isolation among populations. Over time, this generates a clear differentiation between populations of the same species,

causing genetic and even morphological divergences (Allan & Castillo, 2007). Conservation and environmental protection practices can reduce the consequences generated by these problems.

From the data presented with genetic and morphometric evidence, we suggest that population belonging to the Rita stream constitutes a new species within the genus *Psalidodon*, here called *P. rioparanaibanus* n. sp., and must be formally recognized, receiving "endangered" status, due to their endemism.

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New report of shallow water scleractinians from the Pliocene of Siena

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ABSTRACT   Three species of scleractinians belonging to the genera *Hoplangia* Gosse, 1860, *Phyllangia* Milne Edwards et Haime, 1848 and *Thalamophyllia* Duchassaing, 1870 are reported. For *Hoplangia* and *Thalamophyllia* this is the first report from the Mediterranean Pliocene. The three species were linked to hard or detrital bottoms of the infralittoral zone.

KEY WORDS   Early Pliocene; Tuscany, Monte Calcinaio; Scleractinians; Caryophyllidae; *Hoplangia, Phyllangia* and *Thalamophyllia.*

INTRODUCTION

Several contributions to knowledge of the scleractinian fauna of Siena, and in particular to the fauna of Monte Calcinaio in the Siena-Radicofani basin (Tuscany, Siena), were recently published (Spadini, 2015, 2016, 2018). A feature of this population is its quantitative and qualitative species richness, related to hard or detrital bottoms of the infralittoral to circalittoral and, in some cases, bathyal environments.

This note describes three species of the Monte Calcinaio, two belonging to the genera *Hoplangia* Gosse, 1860 and *Thalamophyllia* Duchassaing, 1870. They have no fossil representatives in the Mediterranean Pliocene and are currently represented by one species each in the Mediterranean Sea. The third species belongs to the genus *Phyllangia* Milne Edwards et Haime, 1848, which is represented by various species, living and fossil, one of which (*P. mouchezii*) occurs in the Mediterranean Sea. All these species are linked to hard or detrital bottoms, a type of habitat little known in the Mediterranean Pliocene, but present in that of Siena.

Monte Calcinaio (altitude 732 m; 52°54'55"N, 11°48'59"E), where the materials were found, is near Radicofani in the Radicofani sub-basin (Tuscany, Italy). Marine sedimentation in this area started in the Zanclean with deposition of deep-sea clays over continental Miocene sediments (Bonini & Sani, 2002). The microfaunal association in the Radicofani Basin has been attributed to the *Globorotalia margaritae/puncticulata* Biozone of the Early Pliocene (Zanclean) (Bossio et al., 1992; Pascucci et al., 2006). For other general characteristics of this site, see Spadini (2015, 2016, 2018).

RESULTS

Systematics

Classis ANTHOZOA Ehrenberg, 1834
Subclassis HEXACORALLIA Haeckel, 1866
Ordo SCLERACTINIA Bourne, 1900
Familia CARIOPHYLLIIDAE Dana, 1846

Genus *Hoplangia* GOSSE, 1860

TYPE SPECIES. *Hoplangia durotrix* Gosse, 1860, by monotypy.

REMARKS. *Hoplangia* Gosse, 1860 is only represented by the extant *H. durotrix* Gosse, 1858 that lives in the Eastern Atlantic and the Mediterranean Sea. Cairns (1995) reported this species from New Zealand. For its general characters, see Joubin (1927), as *Microcyathus neapolitanus* Doderlein, 1903, and Zibrowius (1980).

Hoplangia durotrix is linked to rigid substrates, common in coastal areas and coralligenous zones to depths of about 150 m (Zibrowius, 1980).

Hoplangia sp. (Fig. 1)

EXAMINED MATERIAL. Monte Calcinaio: one colony.

DESCRIPTION. Fragment of colonial corallum, bushy, very small in size (17.6 x 8.4 x 11.6 mm) formed by extratentacular budding. The corallites originate from the sides of other corallites. Theca covered with coarse granules, wide costae, not always well defined.

Small, circular or slightly elliptical calices (max. 3.2 x 4.7 mm). Septa 24–36 arranged hexamerally in four incomplete cycles. Fossa very deep. S_1 more robust and thicker than S_2, S_3 converging with S_2 deep in fossa; S_4 small, only present in some of the six systems. Axial edges of septa smooth, but the edges of S_1 and S_2 are slightly wavy in the centre, tuberculated and probably fused together at their base. Lateral faces of septa bear very thick circular granuli. Pali and columella absent.

REMARKS. Due to the general characters, the colony (inner edge of septa smooth and non-toothed, absence of pali and columella) it can be included in the Caryophylliidae family. In addition to the lack of pali and columella, the Siena colony shares the following characters with *Hoplangia:* the large granules of the theca, the costae not well defined, the septal characters with $S_1 > S_2$, the tubercles of the axial margin of the septa fused at the bottom of the calice, and the large granules on the lateral faces of the septa.

Genus *Phyllangia* Milne Edwards et Haime, 1848

TYPE SPECIES. *Phyllangia americana* Milne Edwards & Haime, 1849, by subsequent designation (Milne Edwards & Haime, 1850).

REMARKS. The genus *Phyllangia* was included in the Rhizangidae family, but was recently transferred to the Caryophylliidae by Zibrowius (1980).

The oldest fossils of the genus *Phyllangia* date back to the Oligocene of Albania (Lorenthey, 1926).

Other species have been described for the Miocene of Western Europe (Milne Edwards & Haime, 1848–1850; Michelotti, 1871; Chevalier, 1961), Borneo (Gerth, 1923), Florida (Wells, 1947; Gane, 1900) and Land of Fires (Squires, 1963).

Various species can be found along the Atlantic and Indo-Pacific coasts (Cairns & al., 1999). One of these, *Phyllangia mouchezii* (Lacaze-Duthiers, 1897), present in the Atlantic Ocean and in the Mediterranean Sea (Zibrowius, 1980), is considered a subspecies of *P. americana* Milne Edwards & Haime, 1848 (Chevalier, 1966; Cairns, 2000).

Phyllangia sp. (Fig. 2)

EXAMINED MATERIAL. Monte Calcinaio: one colony.

DESCRIPTION. Colonial corallum composed of two complete and two incomplete corallites. Corallites arising by extratentacular budding from a thick crusty coenosteum or from the lower theca of a parent corallite. Corallites cylindrical, 5–6 mm tall, closely spaced or fused at their base. Theca granular and costate at the main septa. Shallow fossa, circular or slightly elliptical calice (max 12.6 x 9.7 mm). Septa in five incomplete cycles arranged hexamerally, but incomplete and poorly conserved. The largest calice has 52 septa, but is not fully preserved.

S_1 and S_2 equal or sub equal, thicker than the septa of higher cycles, S_3 dilated at their base, forming an indistinct paliform lobe, present only in some septa. Some S_4 seem to have slightly serrated margins, S_5 reduced and not very evident, present in the largest corallite. S_1, S_2 and S_3 merge at bottom of calice, while S_4 and S_5 are free. Lateral face of septa with sharp granules. Columella papillary, very small, composed of few elements. Endothecal dissepiments present.

REMARKS. Among the species described, this colony resembles *Phyllangia blakey* Wells, 1947 from the Upper Miocene of Florida, and *Phyllangia mouchezii,* currently living in the Mediterranean Sea (see Zibrowius, 1980).

De Angelis (1894) described *Phyllangia mi-*

crosyderea, a species with completely different morphological characters from the Pliocene of Albugnano (Asti).

Genus *Thalamophyllia* Duchassaing, 1870

TYPE SPECIES. *Desmohyllum riisei* Duchassaing, 1860, by monotypy.

DIAGNOSIS. Colonial corallum formed by extratentacular budding of ceratoid corallites from a thin common basal coenosteum resulting in reptoid to phaceloid coralla. Pali and columella absent; fossa deep. Endotheca absent (Cairns, 1995).

REMARKS. *Thalamophyllia* is a genus of colonial coral characterized by reptoid colonies, but isolated corallites have often been found (Cairns, 1995).

Four species are recognised: *T. riisei* (Duchassaing et Michelotti, 1864) of the western Atlantic, *T. tenuescens* (Gardiner, 1899) of the Indian Ocean and western and central Pacific, *T. gasti* (Doderlein, 1913) of the Mediterranean Sea and eastern Atlantic, and *T. gombergi* Cairns, 1979 of the western Atlantic (Cairns et al., 1999).

***Thalamophyllia* sp. (Fig. 3)**

EXAMINED MATERIAL. Monte Calcinaio: one specimen.

DESCRIPTION. Corallum conical, elongated, 11.7 mm tall, with a calicular diameter of 2.2 mm, curved distally. Broad base, covered with evident granuli. The six costae corresponding to the septa
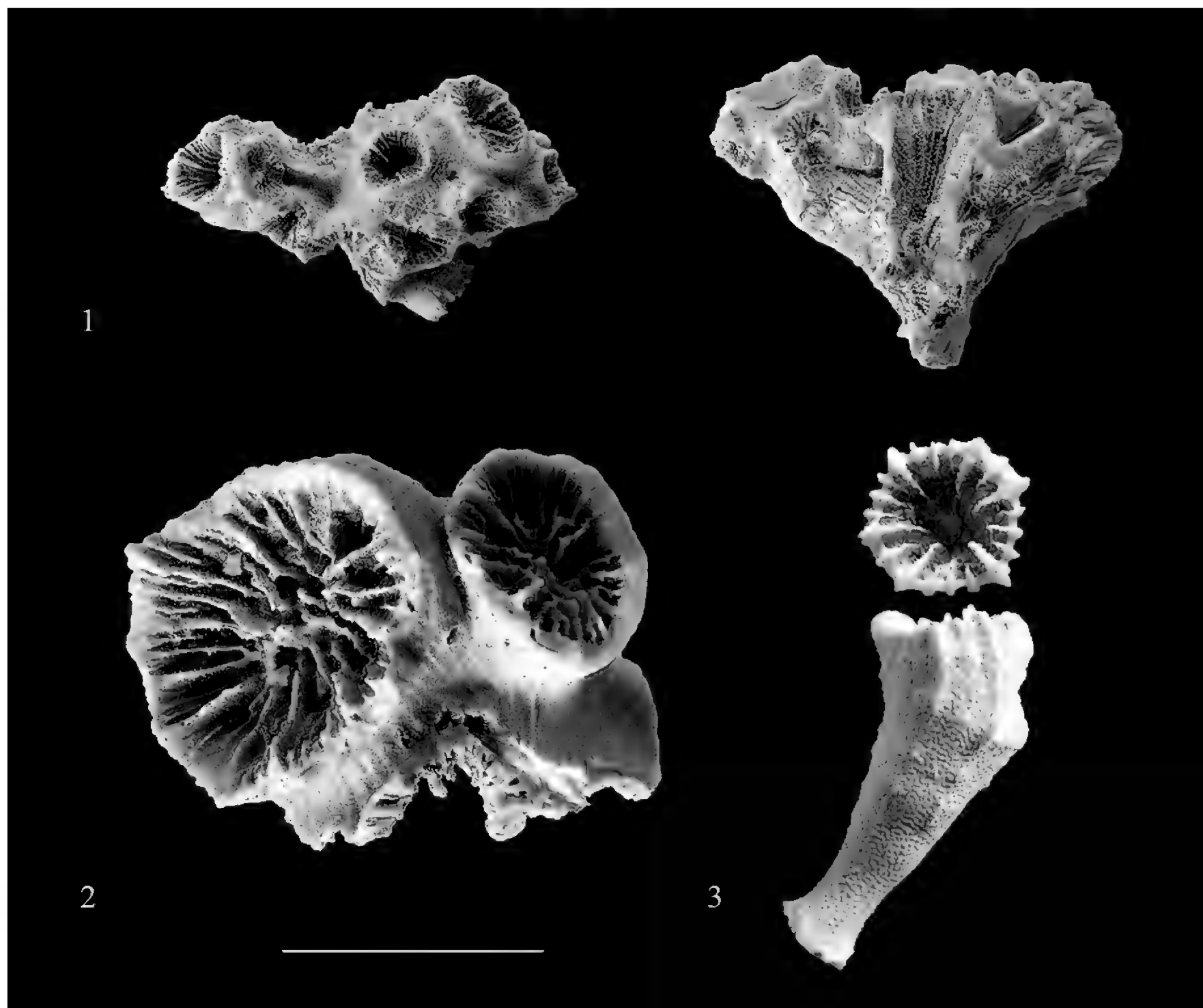
Figures 1–3. Scleractinian of Early Pliocene (Zanclean) of Monte Calcinaio. Fig. 1: *Hoplangia* sp. Fig. 2: *Phyllangia* sp. Fig. 3: *Thalamophyllia* sp. (Scale bar = 1 cm)

of the first cycle are evident from the peduncle. The costae corresponding to the second and third cycle are faintly evident. Calice regularly circular. A total of 20 septa arranged in six systems and three incomplete cycles, with four S_3 missing in two opposing systems.

S_1 robust, reaching half of the calicinal radius, S_2 is thinner, shorter than S_1, and S_3 is even smaller than the previous cycle. Very deep fossa, no columella.

REMARKS. According to the data reported in Zibrowius (1980) and Addamo et al. (2016), young *Desmophyllum* of equal calicinal diameter have more septa than *Thalamophyllia*. From a morphological point of view, the specimen from Monte Calcinaio is similar to *Desmophyllum fasciculatum* (= *T. gasti*) in Joubin (1927) and *Thalamophyllia tenuescens* (Gardiner, 1899) from New Zealand and the Philippines (Cairns, 1995).

Other small-sized specimens have been found on Monte Calcinaio, but they are generally in a very poor state of conservation. All these specimens are characterized by a broad base, three incomplete cycles of septa, theca with granules and costae variously developed.

CONCLUSIONS

The genera *Hoplangia* and *Thalamophyllia,* hitherto known only from the Pleistocene and still living in the Mediterranean Sea, are two new genera from the Mediterranean Pliocene. By contrast, the genus *Phyllangia* is reported since the Miocene, but its presence in the Pliocene was uncertain (Vertino et al., 2014).

The species described show a certain morphological affinity with the corresponding species extant in the Mediterranean and it is therefore likely that they had similar ecological needs (Zibrowius, 1980).

Thalamophyllia has a wide bathymetric range between 25 and 2460 m (Cairns, 2000). *T. gasti* currently lives in the Mediterranean Sea at shallow depths in caves, under small overhangs and in coralligenous environments with *Corallium rubrum*. *Hoplangia* lives between depths of 6 and 150 m (Zibrowius, 1980), while live specimens of *Phyllangia mouchezii* have been found between depths of 1 and 55 m (Zibrowius, 1980), although other species reach greater depths. It is therefore possible to hypothesize that the specimens found on Monte Calcinaio lived at depths between 6 and 55 meters, corresponding to the infralittoral zone.

This bathymetric range matches that of other species typical of hard or detrital bottoms, from Monte Calcinaio, such as *Madracis almerai, Monomyces* sp., *Cladopsammia* sp., sharing the same habitats and is confirmed by various species of gastropods (*Gibbula* sp., *Persististrombus coronatus, Thais hörnesiana*) and bivalves (*Glycymeris bimaculata, Aequipecten scabrellus, Ostrea* sp., *Gigantopecten latissimus, Spondylus crassicosta*) which are found especially in the easternmost part of Monte Calcinaio.

The finding of these three species related to hard bottoms confirms the importance of this site for the study of the scleractinian fauna of the Pliocene of Siena and of the Mediterranean area in general.

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Rediscovery of *Armiger crista* (Linnaeus, 1857) (Gastropoda Planorbidae) in Algeria

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ABSTRACT   *Armiger crista* (Linnaeus, 1758), a holartic freshwater gastropod, has been rediscovered in Algeria. It was found in a small lake whose malacofauna was almost exclusively paleartic/holartic. This aquatic environment is probably unique in the Maghreb, where cold-climate faunal elements are rapidly disappearing due to global warming. Monitoring of the site and study of other groups is highly recommended.

KEY WORDS   *Armiger crista*; endangered paleartic gastropods; global climate change; southern limit.

INTRODUCTION

Armiger crista (Linnaeus, 1758), type locality: marshes in Germany, is a holartic freshwater/α-oligohaline pulmonate gastropod, belonging to the family Planorbidae. The Maghreb and Ethiopia constitute in Africa the southern limit of its range (Van Damme, 1984; Brown, 1994). In the last decades it has only been recorded from a few sites, namely at Ifrane, Lake Hachlaf and the Ben Smim springs in the Middle Atlas in Morocco (Ghamizi, 1998) and from a well at Jandouba, north-west Tunisia (Khalloufi & Boumaiza, 2007).

In Algeria, it was considered as possibly extinct (Van Damme & Ghamizi, 2010, Van Damme et al., 2010), not being recorded anymore since the 19th Century, when Bourguignat (1864) listed it as *Planorbis crista* and *P. imbricatus* (= *P. crista*), from the Mediterranean region between Mostaganem and Constantine (see also Lallemant, 1881).

Subfossils shells have been found in the southern part of the country at Hassi-Dehin and the Ahaggar Mountains (Sparks & Grove, 1961; see also: Thomas 1884: 50–51, tableau synoptique and Pallary, 1901: 160).

In July, 2020, during an inventory survey of the malacological fauna of Algeria, specimens of *Armiger crista* were found in a small lake near Tizi-Ouzou.

MATERIAL AND METHODS

Sampling was carried out in the region of Tizi-Ouzou, about 100 km east of Algiers (Algeria). The site is a natural lake, shallow, with a length of 80 meters and a width of 30 meters, it is rich in vegetation and animals occupying this habitat, located at an altitude of 460 meters with coordinates 36°48'14"N, 4°00'35"E (Fig. 1).

The samples (Fig. 2), a dozen specimens of *Armiger crista*, were taken with a hand net (0.5 mm-mesh size). The shell shows regular transverse ridges extending beyond the main curve of the shell. It is translucent, yellow-brownish in colour and has had a maximum diameter 1.83–2.19 mm.

Other additional species were found in this place, such as *Hippeutis complanatus* (Linnaeus, 1758), *Bulinus truncatus* (Michaud, 1829), *Ferrissia californica* (Rowell, 1863), *Musculium lacustre* (O.F. Müller, 1774), and *Planorbis planorbis* (Linnaeus, 1758).

DISCUSSION AND CONCLUSIONS

Since the late 19th Century little field-work has been carried out on the freshwater malacofauna of Algeria. Nonetheless, it is evident that, due to the ever intensifying and unsustainable human use of surface waters, many of these have disappeared or become devoid of aquatic life since that time. In addition, increasing ambient temperatures and extended periods of drought, due to climate change, have in the last decades aggravated this ecological decline (Oualkacha et al., 2017). In all Maghrebian countries, the freshwater gastropod fauna in general has severely suffered, but in particular the paleartic-holartic species, whose range's southern limit was situated in northern Africa and the Levant. These are presently vanishing all over their historical southern range (Seddon et al., 2014).

The discovery in Algeria of a standing surface water, where *H. complanatus* L. was found (Glöer & Ramdini, 2019), harboring not only *Armiger crista*, considered as endangered, but harboring a mollusc community consisting almost exclusively of holartic molluscs, *Bulinus truncatus* excepted, is of substantial scientific importance. It indicates that such aquatic relict environments still exist in the Maghreb, though having become extremely rare. It is therefore highly recommended that the small lake in question should be protected, considering its ecological uniqueness and that a monitoring program should be set up to track changes in abiotic and biotic (e.g., molluscs) parameters over time in function of global warming trends in the Maghreb.

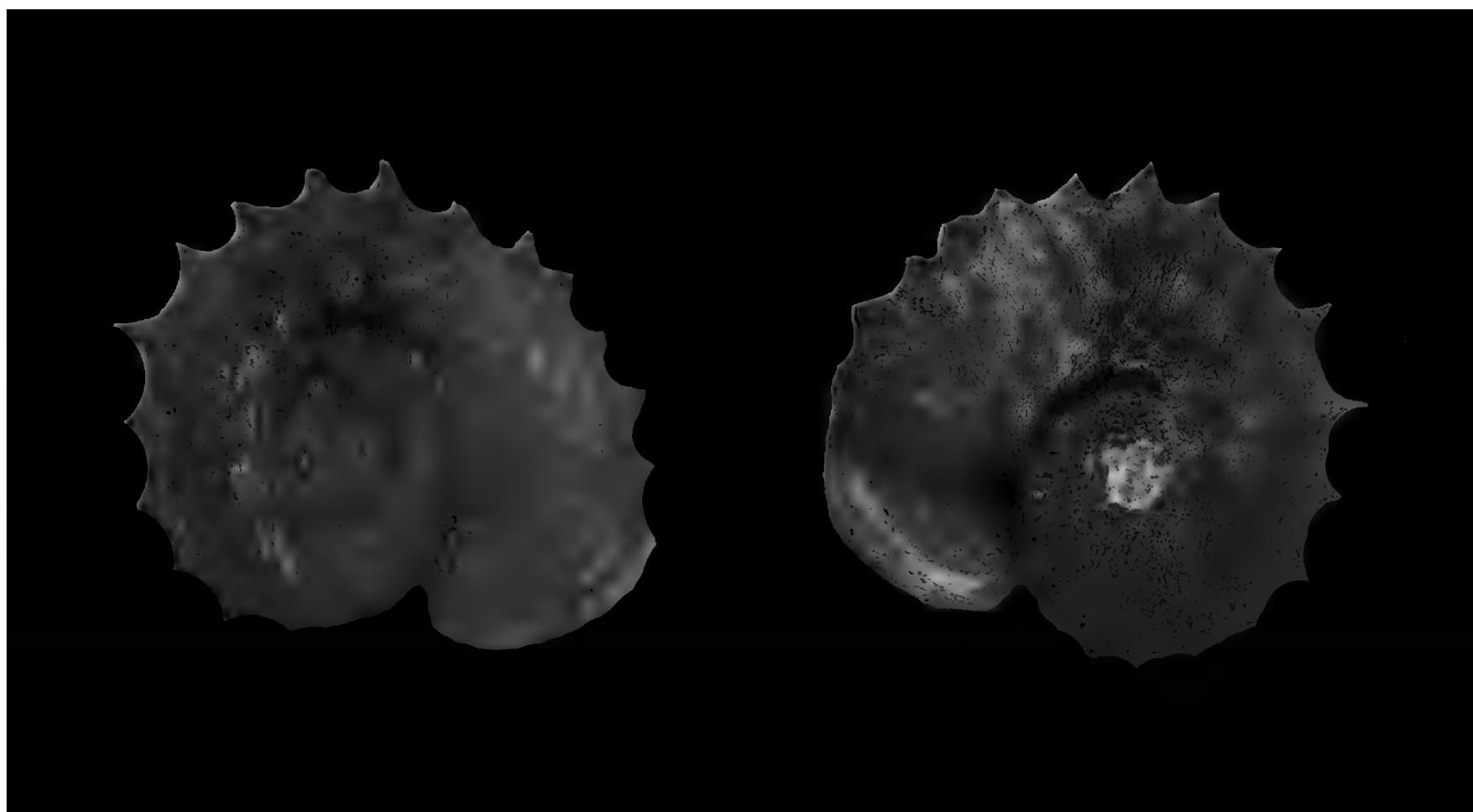Figure 1. Small lake near Tizi-Ouzou (Algeria).

Figure 2. *Armiger crista* from Tizi-Ouzou (Algeria), maximum diameter 1.94 mm.

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An overview of the Hymenoptera Chrysididae from humid urban zones near Abu Dhabi (UAE)

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ABSTRACT   The Chrysididae Hymenoptera of the Abu Dhabi territory has ben studied by means of Malaise Traps. The comparison between humid and dry zones reveals the richer biodiversity preserved even in small humid zones. Two new species are discovered and described, namely: *Hedychridium caerulescens* n. sp. and *Chrysis houbaraeensis* n. sp. In the humid zones the uncommon *Adelopyga huberi* Kimsey, 1988 is discovered and illustrated.

KEY WORDS   Chrysididae; cuckoo wasp; United Arab Emirates; Abu Dhabi; humid zones.

INTRODUCTION

Abu Dhabi (UAE) is the westernmost town of the United Arab Emirates (UAE). Located along the flat and sandy shores on the Persian Gulf, it is surrounded by seawater and marshes. Humid zones near Abu Dhabi were recently established as nature reserves to protect the unique biodiversity.

Recently we have investigated the effects of humid zones on the insect's biodiversity (Strumia et al., 2017). Similar results were also observed in Arabia: Oman, Dophar Province (F. Strumia unpublished data). The availability of permanent water around all the year allows the presence of a local rich and diversified insects population, especially in a dry land.

The largest species number was observed in the most studied humid sites confirming the result recently observed in the Mediterranean region (Strumia el al., 2017).

MATERIAL AND METHODS

Insects were captured in two nature reserves near Abu Dhabi (UAE) by using Malaise traps, namely:

1 - "Al Wathba Wetland Reserve". The trap was operated by A. Saji & A. van Harten and captured from February 2015 up to April 2017. Al Wathba Wetland Reserve, with a surface of about 4.9 square km, is a complex of permanent surface water bodies, both natural and man made, at about 40 km south-east of central Abu Dhabi. Al Wathba was established as a nature reserve in 1998. The soil is mainly dry and sandy, thus favorable to the nesting of several Hymenoptera species (Figs. 1, 2).

2 - "Houbara Protected Area": A. Saji & A. van Harten operated a Malaise trap. Insects were captured in this reserve from November 2015 up to April 2017.

Houbara Protected Area is located in the Al Dafra region, south of road E11 with a surface of about 770 square km. The reserve consists of a costal plain of dry sandy ground and gravel terrains (Fig. 3). The proximity to the Persian Gulf coast

provides some humidity. Houbara Protected Area was established in 2014.

While "Al Wathba Wetland Reserve" is a truly humid site inside the urban area, the "Houbara Protected Area" is located about 120 km west of the Abu Dhabi town and along the Persian Gulf coast. Houbara appears to be a standard dry biotope in Arabian Desert. Thus it is possible to perform a comparison with the material captured in the humid "Al Wathba Wetland Reserve".

A few additional Chrysididae (about 12% of the total) were randomly captured around Abu Dhabi during this investigation, mainly in dry sites Southeast of town (Fig. 4).

These specimens are represented by common species widely distributed into Arabian Peninsula and typical of the Near East Chrysididae fauna (Strumia & Dawah, 2008; Strumia, 2014). Surprisingly, three *Hedychridium* resulted to belong to a new species. Body shape and color differ from all other Near East *Hedychridium*. This novelty is worth to be described here.

RESULTS

In the above two Protected Areas between 2015 and 2017 the Malaise traps captured about 131 Hymenoptera Chrysididae individuals, that resulted to belong to 22 species and nine genera, as listed below (Table 1).

Systematics

Ordo HYMENOPTERA Linnaeus, 1758
Superfamilia CHRYSIDOIDEA Latreille, 1802
Familia CHRYSIDIDAE Latreille, 1802
Subfamilia CHRYSIDINAE Latreille, 1802
Genus *Adelopyga* Kimsey, 1988

Adelopyga huberi Kimsey, 1988

MATERIAL STUDIED. Houbara Protected Area, 01–30.05.2016, 1 female, Malaise trap.

DISTRIBUTION. Oman (Whaiba sands, Kimsey, 1988). New record for UAE.

Figure 1. View of Al Wathba Wetland Reserve, Abu Dhabi. Figure 2. Aerial view of the Al Wathba Wetland Reserve, Abu Dhabi. Figure 3. Overview of the Houbara Protected Area. Figure 4. Distribution of the protected areas around Abu Dhabi.

N°	Species observed	Al Wathba	Houbara	Near Abu Dhabi
1	*Adelopyga huberi* Kimsey, 1988		#	
2	*Cephaloparnops denticulatus* Spinola, 1838		#	
3	*Chrysis blanchardi* Lucas, 1849	#		Al Bida'a
4	*Chrysis houbarensis* n. sp.		#	
5	*Chrysis jousseaumei* Buysson, 1897	#	#	
6	*Chrysis laeta* Dahlbom, 1854	#		
7	*Chrysis palliditarsis* Spinola, 1838	#	#	
8	*Chrysis viridissima* Klug, 1845	#	#	Al Bida'a
9	*Hedychridium aegyptiacum* Du Buysson, 1898	#		Al Bida'a
10	*Hedychridium aequabilitum* Linsenmaier, 1994	#		
11	*Hedychridium anithae* Strumia, 2014	#	#	Al Bida'a
12	*Hedychridium caerulescens* n. sp.			Al Tawi
13	*Hedychridium disiunctum* Linsenmaier, 1994		#	
14	*Hedychridium holopygum* Linsenmaier, 1994	#		
15	*Hedychridium iridirufum* Linsenmaier, 1994	#	#	Al Tawi
16	*Hedychridium planifrons* Du Buysson, 1900	#		
17	*Hedychrum alfierii* Trautmann 1926		#	
18	*Holopyga beaumonti* Balthasar, 1953	#		
19	*Holopyga subglabrata* Linsenmaier, 1994	#		
20	*Holopyga vicissituda* Linsenmaier, 1994	#		
21	*Omalus margianus* (Semenov, 1932)	#	#	
22	*Platycelia ehrenbergi* Dahlbom, 1845	#		
23	*Spintharina dubai* Bohart, 1987	#		
24	*Spintharina integerrima* Klug, 1845			Al Ramlah
	N° of species	**17**	**11**	**7**

Table I. Checklist of Chrysididae species observed near Abu Dhabi reserves in 2015–2018.

REMARKS. *Adelopyga* was described as a highly modified genus of Elampini (Chrysididae). Until now *A. huberi* was known from two females from south Oman. This second find confirms its presence in western Arabian Peninsula. The holotype has: "*abdominal sterna flat or shallowly convex ... It should be noted that the type and paratype specimens were preserved in alcohol and then critical point dried. The convexity of the abdominal sternum may be due to this treatment*" (Kimsey, 1998: 334). It is worth to note that the specimen from Abu Dhabi has a concave sternum as usual of all the Chrysididae species (Fig. 5). Our find shows that "*abdominal sterna flat or shallowly convex*" is not a distinctive feature of *Adelopyga* genus.

Cephaloparnops Bishoff, 1910

Cephaloparnops denticulatus (Spinola 1838)

MATERIAL STUDIED. Houbara Protected Area, 01–30.06.2016, 4 males, 3 females, same locality, 01–30.05.2016, 2 males, Malaise trap.

DISTRIBUTION. Palaearctic, North Africa, Middle East. Iran (Kimsey & Bohart, 1991). New record for UAE (Strumia, 2014).

Figure 5. Female of *Adelopyga huberi* Kimsey, 1988; above: dorsal view; below: lateral view; right: head and dorsum.

Chrysis Linnaeus, 1761

Chrysis blanchardi Lucas 1849

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 04.04.2017, 4 males; same locality, 20.04. 2017, 1 male, Malaise trap.

DISTRIBUTION. North Africa, Spain, (Linsenmaier, 1999).

Chrysis houbaraeensis n. sp. Figs. 6–9
http://zoobank.org/urn:lsid:zoobank.org:act:7EF2 54F5-05E3-44A8-A28F-C3629CC1A617

DIAGNOSIS. The male entirely blue, the bicolor female, the shape of third Tergite, the head and pronotum trasverse, larger then longer, identify the new species.

EXAMINED MATERIAL. Holotype male: UAE, Abu Dhabi, Houbara Protected Area, 01–30.06.2016, Malaise trap, leg. A Saji & van Harten. The holotype is preserved in the Natural History Museum of Pisa University. Paratypes: 1 male, same locality, 01–30.05.2016, Malaise trap, leg. A Saji & A. van Harten; 1 male, same locality, 01.11–10.12.2015, Malaise trap, leg. A Saji & A. van Harten; 1 female, same locality, 01–30.05.2016, Malaise trap, leg. A. Saji & A. van Harten.

DESCRIPTION OF HOLOTYPE MALE. Body length: L=5.6 mm. Body color: uniform blue in color.

Metasoma Sternum II is metallic blue with rounded well-separated black spots in contact with lateral border.

Head. Transverse, larger then longer: L/W = 0.43; scapal basin with white decumbent setae (Figs. 5, 6); TFC strong with two backward arms surrounding the middle ocellus; malar space about 1 MOD long; clypeus in middle elevated with a black distal border; ocellocular angle obtuse; distance between lateral ocellus and compound eye larger than one MOD; mandible black, distally brown.

Mesosoma. Pronotum short, transverse with pointed anterior angles (Figs. 5, 6); punctures on head and pronotum deep, about 0.4 to 0.6 MOD in

diameter; punctures separation less then their diameter; a few very small (0.2 MOD or less) punctures between; punctures stronger and separated as much as their diameter (Figs. 5, 9) on mesonotum central part and scutellum; legs metallic green with tarsus non-metallic clear brown; mesopleuron punctures smaller than on Mesonotum; hairs white and short on all body; distal border of third tergite has a very thin transparent rim; tegula unusually small (1.7 MOD long) and metallic blue.

VARIABILITY. Body length: male paratype: L=6.0 mm and 6.1 mm respectively. female paratype, L= 6.4 mm. Males are uniform blue in color, the female paratype is bicolor: blue green body with mesonotum and scutellum red between punctures but with punctures bottom green (Fig. 9). Female has metasoma with strong red shining on disc of TII and TIII

(Fig. 7). Sexual dimorphism: the difference between males and the only one female available is mainly in the red color on Mesosoma and Metasoma.

DISTRIBUTION. New record for UAE.

ETYMOLOGY. The species is named from the collecting site.

REMARKS. The new species belong to the *C. pulchella* species-group, as demonstrated by the male genitalia (Fig. 8) and the shape of third tergite (Figs. 6, 7).

Chrysis (Exachrysis) jousseaumei Buysson 1897

EXAMINED MATERIAL. Houbara Protected Area, 01–30.05.2016, 3 females; same locality, 01–30. 06.2016, 2 females; same locality, 01–30.04.2016, 2
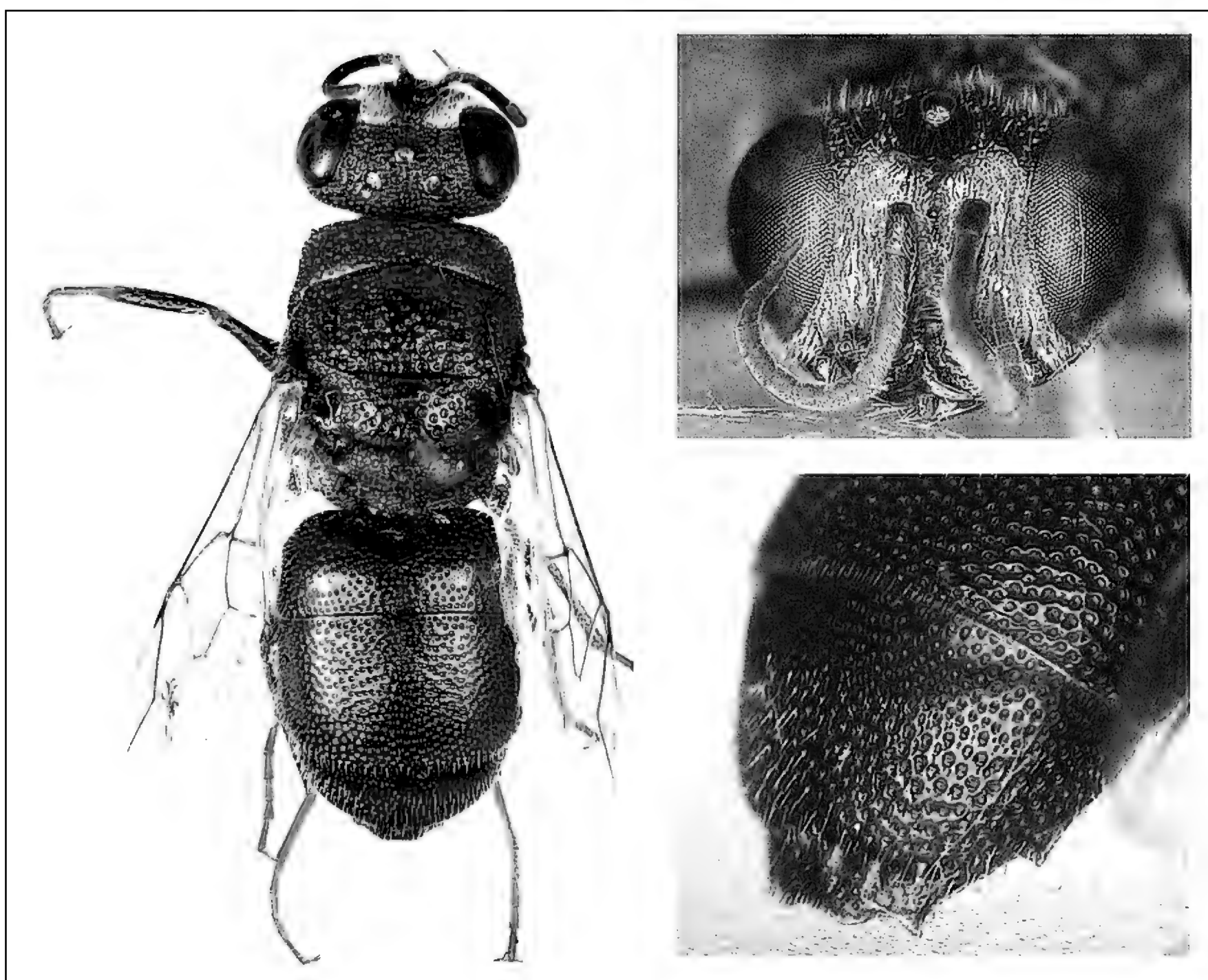

Figure 6. Male holotype of *Chrysis houbaraeensis* n. sp. Mesonotum dorsal view, face in frontal view (right above), Metasoma distal border and Tergum 3 in top lateral view (right below).
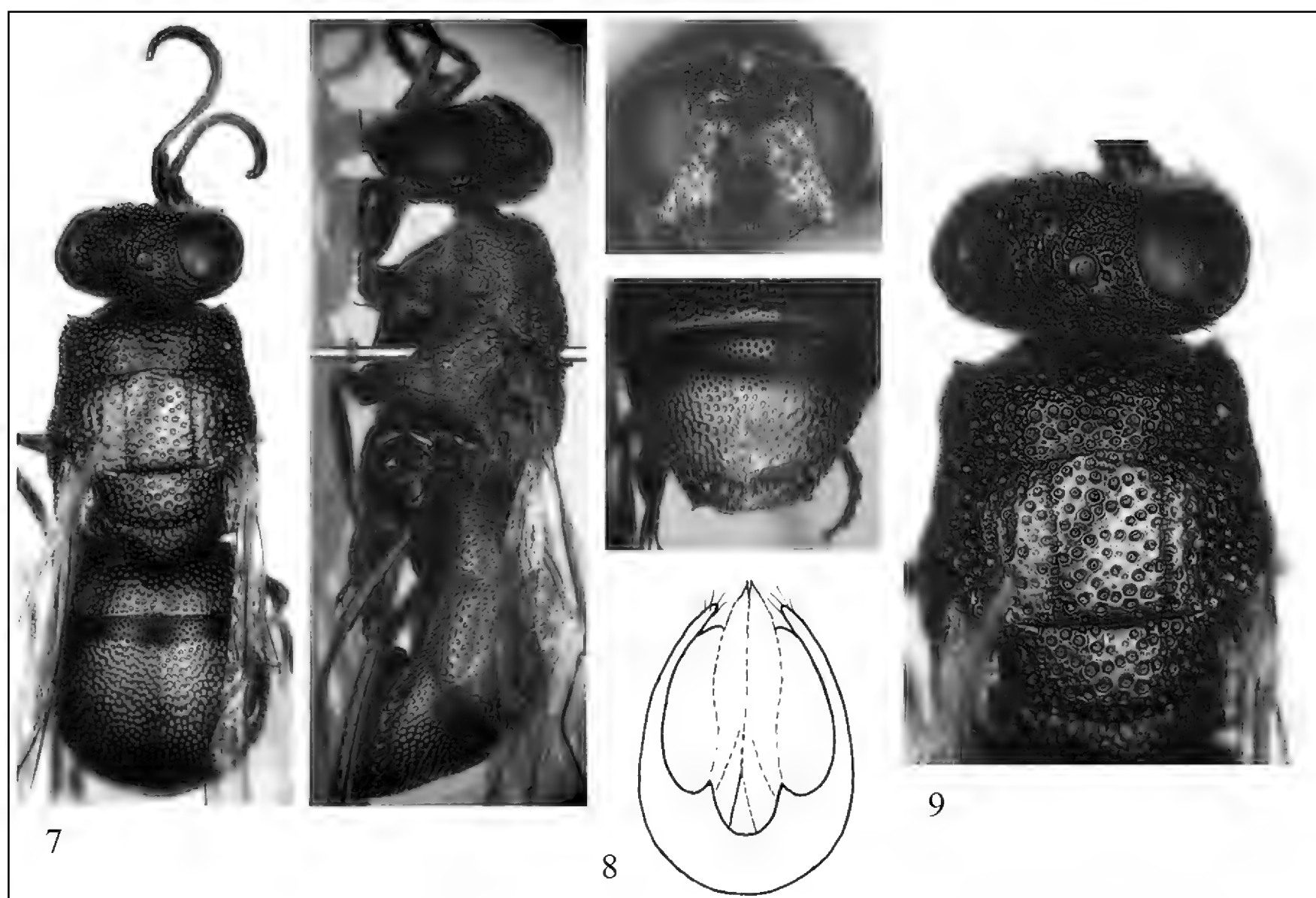
Figure 7. Female paratype of *Chrysis houbaraeensis* n. sp.; dorsum top view and lateral view; face, frontal view; metasoma Tergum 3 distal border. Figure 8. Outline of male genitalia of *Chrysis houbaraeensis* n. sp., holotype in the ventral view, length: 0.07mm. Figure 9. Detail of mesosoma dorsum of *Chrysis houbaraeensis* n. sp. paratype female.

females; same locality, 1.11–10.12.2015, 1 female; same locality, 18.05.2017, 2 females. Al Wathba Wetland Reserve, 10.05.2017, 1 male.

DISTRIBUTION. Northern half of Africa, Yemen, Somalia, UAE (Linsenmaier, 1994; Strumia, 2014).

Chrysis laeta Dahlbom, 1854

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–28.02.2015, 1 female, Malaise trap.

DISTRIBUTION. Algeria, Tunisia, Egypt, Oman, Yemen. UAE (Strumia, 2014).

Chrysis palliditarsis Spinola, 1838

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 10.05.2017, 3 males, 1 female; Houbara Protected Area, 01.11–10.12.2015, 6 males; same locality, 01–30.04.2016, 1 male; Al Wathba Wetland Reserve, 01–31.03.2016, 5 males; same local-ity 19.09–31.10.2015, 3 males; same locality, 01–31.01.2015, 1 male.

DISTRIBUTION. North Africa: Egypt, Ethiopia, Eritrea, South Africa, Asia: Palestine, Oman, Iran, Saudi Arabia, Yemen, and U.A.E. (Linsenmaier, 1994). *Chrysis palliditarsis* is a widespread and common species of the South Palaearctic Region.

Chrysis viridissima Klug, 1845

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–31.03.2015, 1 female; same locality, 01-31.07.2015, 4 females; same locality, 01–28. 02.2015, 1 female; same locality, 19.09–31. 10.2015, 5 females; same locality, 04.04.2017, 2 fe-males, 1 male; same locality, 10.05.2017, 1 female; Houbara Protected Area, 01–30.04.2016, 1 female; same locality, 01.11–10.12.2015, 1 female.

DISTRIBUTION. Afrotropical Region. North Africa: Algeria, Egypt, Libya, Mauritania. Asia:

India, Saudi Arabia, UAE, Oman, Iran, Turkey, Palestine (Linsenmaier, 1994; Madl & Rosa, 2012).

Genus *Hedychridium* Abeille de Perrin, 1878

Hedychridium aegyptiacum Du Buysson, 1898

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–31.01.2015, 2 males, 4 females; same locality, 01–28.02.2015, 2 males, 6 females; same locality, 01–31.03.2015, 2 males; same locality, 04.04.2017, 1 male.

DISTRIBUTION. Egypt, Morocco, UAE (Strumia, 2014).

Hedychridium aequabilitum Linsenmaier, 1994

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–28.02.2015, 2 males, 2 females; same locality, 01–31.01.2015, 3 males.

DISTRIBUTION. Saudi Arabia, Oman, UAE (Linsenmaier, 1994; Strumia, 2014).

Hedychridium anithae Strumia, 2014 (= *Hedychridium cyaneum* Linsenmaier, 1999 ?). Fig. 10

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–28.02.2015, 2 females; same locality, 01–31.01.2015, 1 female; same locality, 01–31.03. 2015, 1 male, 1 female; Houbara Protected Area, 01–30.05.2016, 1 female.

DISTRIBUTION. Iran, Southern-Africa, UAE and Tunisia (this last data is doubtful and need to be confirmed).

REMARK. *Hedychridium anithae* was described and illustrated in 2014 (Strumia 2014: 475, plates 11–14).

A paratype of *H. anithae* (also captured in Al Wathba Wetland Reserve) seems to be consistent with the description of *Hedychrum cyaneum* (Brullé, 1846) as proposed by Linsenmaier (1999). *Hedychrum cyaneum* was described by Brullé (1846) from South Africa, Cape of Good Hope. Unfortunately, the type of the species is probably lost and the generic placement is uncertain (Madl & Rosa, 2012, Rosa & Zai-fu Xu, 2015). In 1889, Radoszkowski described another species of *Hedychrum* with the same name [*H.*

cyaneum] from Siberia, thus creating a primary homonymy. Kimsey & Bohart (1991: p. 220) confirmed the placement of the Brullé species in the genus *Hedychrum*. Later, Linsenmaier (1999) assigns the Brullé species to the genus *Hedychridium* and briefly redescribes it, without specifying the specimens he studied, or if he was able to study the type. It is very likely that this was a personal interpretation of an old species, to which he put in synonym *Hedychridium flavipes* var. *cyanomaculatum* Trautmann, 1927. The latter species does not even belong to the genus *Hedychridium*, being, in reality, a *Colpopyga flavipes flavipes* (Eversmann, 1858) (Rosa, 2017), and this makes the proposal of Linsenmaier even more doubtful. In conclusion, for what was said previously, we do not deem that our specimens can be assigned to the supposed *Hedychridium cyaneum* Brullé. We therefore believe that *Hedychridium anithae* Strumia 2014 is a valid species and that
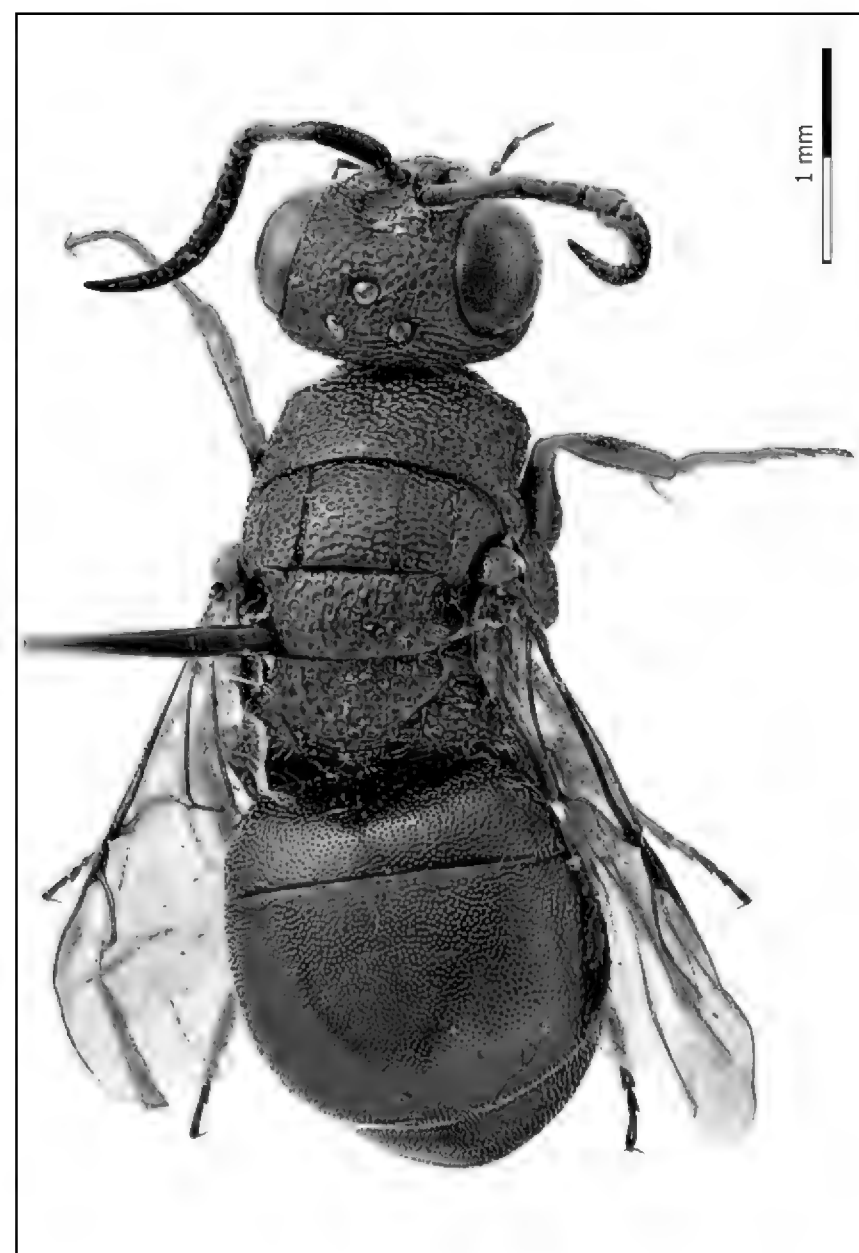

Figure 10. Dorsal view of *Hedychridium anithae* Strumia, 2014, male from Al Bida'a Protected area (Abu Dahbi), 20.07.2018.

Hedychridium cyaneum as described by Linsenmaier (1999) is an inappropriate interpretation of an old description.

Hedychridium anithae is a frequent species in UAE and Iran (Kerman Province: Jiroft, Kahnuj-Tomgoran, Manoojan-Chermil 2017, males and females, leg. M. Purrezalai).

Hedychridium caerulescens n. sp. Figs. 11–13

http://zoobank.org/urn:lsid:zoobank.org:act:505211EA-4A15-4341-B56D-3D123B30B2AA

DIAGNOSIS. The metasoma color distribution, the non-metallic legs and the fine striation between punctures of mesopleuron (Fig. 13) easily identify the new species.

EXAMINED MATERIAL. Holotype female, UAE, Abu Dhabi, Al Tawi Protected Area, 19.09.2018, Malaise trap, leg. A. Saji & A. van Harten. Al Tawi Protected Area. The holotype is preserved in the Natural History Museum of Pisa University. Paratype male: UAE, Abu Dhabi, Al Tawi Protected Area, 19.09.2018 Malaise trap, leg. A. Saji & van Haten. Paratype female: Al Bida'a Protected Area, 29.10.2018, leg. A. van Harten.

DESCRIPTION OF THE HOLOTYPE FEMALE. Body length: L = 3.9 mm. Body color: head and mesosoma metallic blue-green; antenna scape dark brown with Flagellomeres F1 an F2 non-metallic brown. First tergite blue metallic, second also blue metallic, but with a large non metallic brown spot on disc; third tergite blue metallic only in the distal half (Fig. 11).

Head. Larger than longer in top view: L/W = 1.7 Face transverse, scapal basin larger than higher, finely and horizontally striated in middle; mandible partially brown medially with an additional tooth on internal border; gena short, convergent, less than one MOD long; ocellocular angle obtuse; distance between hind acellus and compound eye larger than acellus diameter.

Mesosoma. Punctures on mesonotum relatively small and partially aligned transversally in the central part, larger on scutellum; mesopleuron rounded below with a very fine horizontal striation between the sparse punctures, (Fig.13); tegula small non metallic brown, as tybia and tarsus, femur blue metallic, hind tarsus first joint shorter than followings; antenna last joint long and pointed; wings radial cell close with a weakly sclerotized vein.

Metasoma. Terga punctures very fine and dense; third tergite blue metallic only in the distal half and with a thin transparent rim on distal border, less than 1 PD long, punctures even finer simple, a little larger distally. Metasoma underside brown with a weak blue spot on second sternite.

VARIABILITY. Body length. Male paratype, L= 4.1 mm; female paratype L = 3.9 mm. Sexual dimorphism: minimal in body shape. Male paratype has the first metasoma tergite metallic blue. In female paratype this is non-metallic brown as tybia and tursus; female fore femur nonmetallic distally (Fig. 12); female third tergite weakly more pointed than male. Note that only three individuals are available for comparison.

ETYMOLOGY. The name refers to the blue color of distal part of metasoma terga.

DISTRIBUTION. New record for UAE. The type locality of Al Tawi covers about 45 square km south of Houbara Protected Area and it is a desert dry zone.

REMARKS. In the revision of North-Africa Chrysididae, Linsenmaier (1999) proposed to split the genus *Hedychridium* in group of species. Among these, the roseum-sculpturatum group is well defined and easy to identify. According to Linsenmaier this group is unique by having the first joint of hind tarsus of reduced length, antenna strong and pointed, metasoma punctures fine, body hairs short and white. Several species show more or less non-metallic metasoma terga and legs. Non-metallic metasoma terga were previously associated with species of the *H. roseum* group. The combination of the above features makes identification easy and unquestionable.

The *roseum-sculpturatum* group is species rich and need a new revision of the Palaearctic fauna, since past descriptions are poor and may refer to different taxa. We had recently the opportunity to study a large number of individuals of roseum-sculpturatum group from the Near East (Egypt, Palestine, Syria, Turkey, UAE, Arabia, Yemen and Iran) but none approach the present specimens from Abu Dhabi. It is thus worth to describe them as a new species.

Hedychridium disiunctum Linsenmaier, 1959

EXAMINED MATERIAL. Houbara Protected Area, 01–30.06.2016, 2 females.
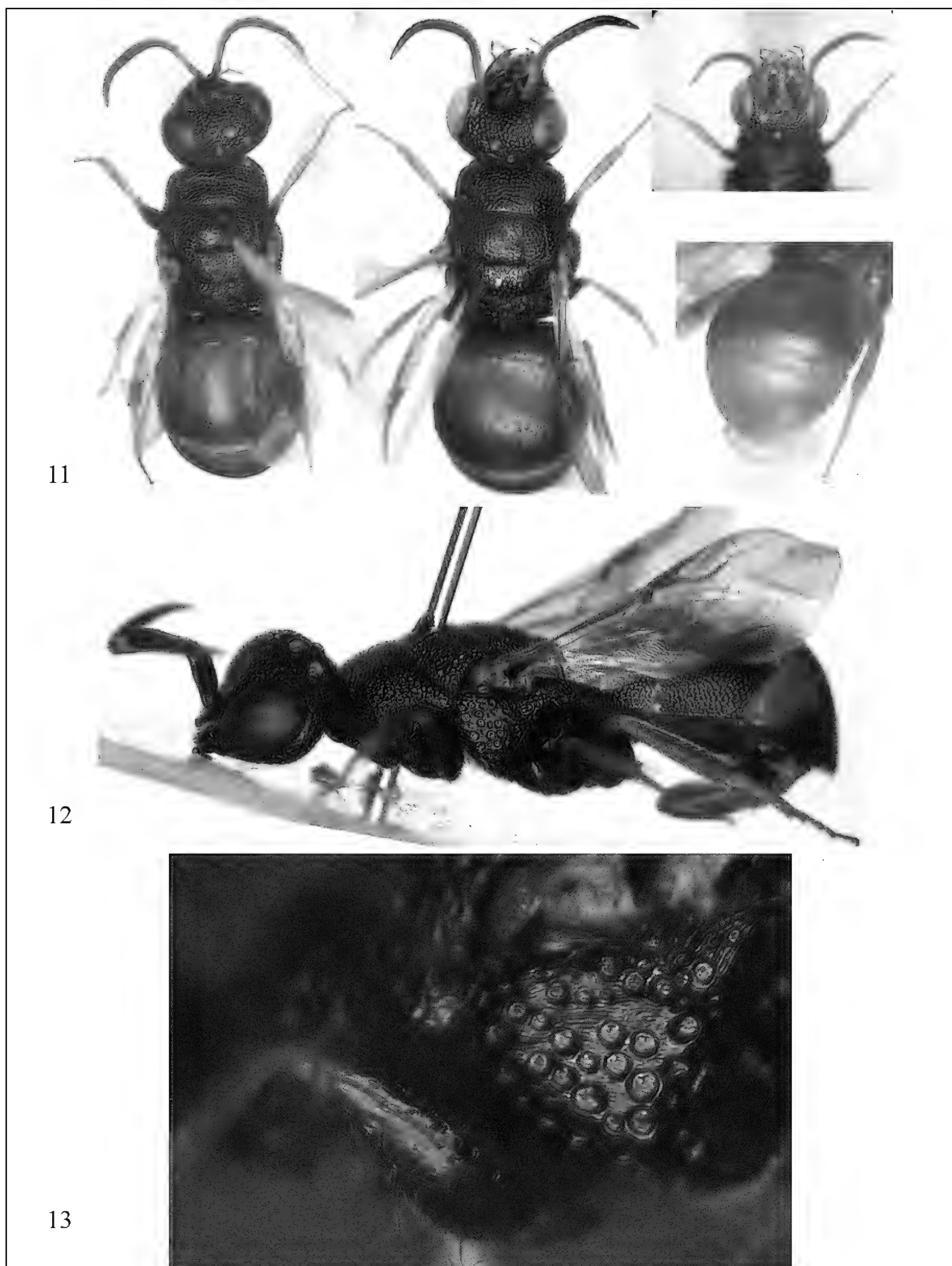
Figure 11. *Hedychridium caerulescens* n. sp.; left: dorsal view of female type; middle: dorsal view of male paratype; right top: face of male paratype; right bottom: posterior side view of male paratype metasoma. Figure 12. *Hedychridium caerulescens* n. sp.; lateral view of female type. Figure 13. Detail of left mesopleuron and fore femur of female paratype of *Hedychridium caerulescens* n. sp.

DISTRIBUTION. Egypt, Mali, Morocco, Saudi Arabia, UAE (Strumia, 2014).

Hedychridium holopygum Linsenmaier, 1994

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–28.02.2015, 3 females; same locality, 01–31.03.2015, 2 males; same locality, 01–31.01.2015, 1 female.

DISTRIBUTION. Qatar, UAE (Strumia, 2014).

Hedychridium iridirufum Linsenmaier, 1994

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 19.09-31.10.2015, 2 males, 1 female; same locality, 01–31.07.2015, 5 males, 8 females; Houbara Protected Area, 01–30.06.2016, 4 females.

DISTRIBUTION. Qatar, Oman, UAE (Linsenmaier, 1994; Strumia, 2008).

Hedychridium planifrons Du Buysson, 1900

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–31.03.2015, 1 female.

DISTRIBUTION. Morocco, Egypt, Palestine, Syria, Oman, UAE (Linsenmaier, 1994).

Genus *Hedychrum* Latreille, 1802

Hedychrum alfierii Trautmann, 1926

EXAMINED MATERIAL. Houbara Protected Area, 01–30.06.2016, 1 male.

DISTRIBUTION. North Africa, Egypt, Palestine, Oman, Saudi Arabia, UAE, Yemen (Strumia, 2014)

Genus *Holopyga* Dahlbom, 1845.

Holopyga beaumonti Balthasar, 1953

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–28.02.2015, 5 males, 2 females; same locality, 01–31.03.2015, 1 male; same locality, 01–31.01.2015, 1 female.

DISTRIBUTION. Iran, Palestine, Saudi Arabia, UAE and Yemen (Strumia et al., 2016).

REMARKS. Linsenmaier (1994) erroneously con-

sidered *H. beaumonti* to be a synonym of *H. colonialis* Mocsáry, 1912. This synonymy is wrong because *H. colonialis* does not belong to the genus *Holopyga* but to the genus *Haba* Semenov, 1954 (Strumia, 2016).

Holopyga subglabrata Linsenmaier, 1994

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01-31.01.2015, 1 male, 1 female.

DISTRIBUTION. Saudi Arabia, Oman, UAE, Yemen (Linsenmaier 1994, Strumia, 2014).

Holopyga vicissituda Linsenmaier, 1994

MATERIAL STUDIED. Al Wathba Wetland Reserve, 01–31.01.2015, 1 male, 1 female.

DISTRIBUTION. Iran, Saudi Arabia, Oman, UAE (Linsenmaier, 1994).

Genus *Omalus* Panzer, 1801

Omalus margianus (Semenov, 1932)

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 04.04.2017, 6 females; same locality, 20.04.2017, 3 females; same locality, 01–31.01.2015, 2 males, 5 females; same locality, 01–28.02.2015, 2 males, 3 females; same locality, 01–31.03.2015, 2 males; same locality, 01–30.04.2015, 1 female; Houbara Protected Area, 01–31.03. 2016, 2 females.

DISTRIBUTION. Iran, Turkmenistan, Uzbekistan (Farhad et al., 2018).

Genus *Platycelia* Dahlbom, 1845

Platycelia ehrenbergi Dahlbom 1845

EXAMINED MATERIAL. Al Wathba Wetland Reserve, 01–30.04.2015, 1 female.

DISTRIBUTION. Algeria, Tunisia, Libya, Morocco, UAE (Linsenmaier, 1999).

Genus *Spintharina* Semenov, 1892

Spintharina dubai Bohart 1987

EXAMINED MATERIAL. Al Wathba Wetland Re-

serve, 01–30.04.2015, 1 male; same locality, 01–30.05.2016, 1 male.

DISTRIBUTION. Iran, Oman, Saudi Arabia, UAE, and Yemen (Linsenmaier, 1994; Strumia, 2008, 2014).

Spintharina integerrima (Klug, 1845)

EXAMINED MATERIAL. Al Ramlah Protected Area, 08.11.2018, 2 females.

DISTRIBUTION. NW-Africa, Palestine, Oman, Saudi Arabia, Sudan, UAE, Yemen.

RESULTS

About 150 individuals of Chrysididae were captured mainly by the Malaise traps with a total of 24 species (Tab. I). Five species resulted to be new records for UAE, namely: *Adelopyga huberi* Kimsey, 1988, *Hedychridium caerulescens* n. sp., *Chrysis houbaraeensis* n. sp., *Chrysis blanchardi* Lucas, 1849, and *Cephaloparnops denticulatus* Spinola, 1838.

The Al Wathba Wetland Reserve has a notable larger richness (17 species, about 36% of known UAE Chrysididae) with respect to the Houbara Protected Area (11 species). This result confirms the larger biodiversity preserved into humid zones, as was recently observed in a Mediterranean biotope (Strumia et al., 2017).

The two Protected Areas share only five species (all widespread species of the Arabian Peninsula, Table 1). Two interesting and uncommon species were observed only in the Al Wathba Wetland Reserve: *Adelopyga huberi* Kimsey, 1988 (new record for UAE) and *Cephaloparnops denticulatus* Spinola 1838 (new record for UAE). Five species were captured only in the Houbara Protected Area and are probably species better adapted to dry biotopes.

Our results confirm the notable richness of genus *Hedychridium* in Arabian region (*Hedychridium caerulescens* n. sp.).

Phenology in Abu Dhabi

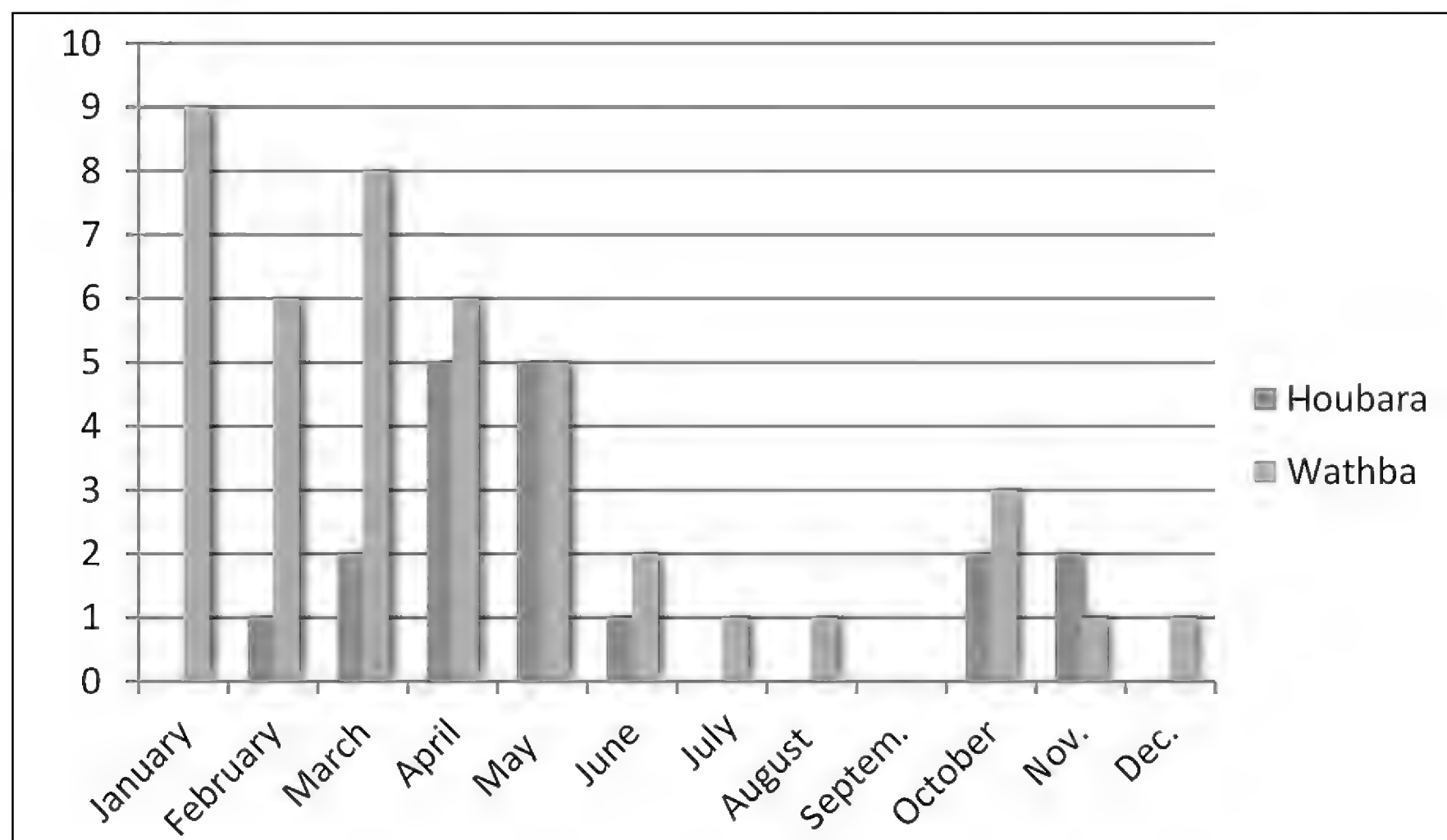The material captured was collected with a monthly timing: only the captures of November

Figure 14. Number of Chrysididae species captured each month in the "Al Wathba" and "Houbara" reserves, respectively.

and December were cumulated. The monthly number of species reveals an interesting and noteworthy different phenology between the two reserves as shown in figure 14. In Al Wathba the largest number of species is observed in early spring followed by a large decrease in summer and autumn. On the contrary in Houbara the maximum species richness is observed between March and June and in November-December. The availability of meteorological data could suggests an interpretation of this data and possible correlations that need to be confirmed by further observations. The phenology of Al Wathba appears to be correlated with the rainfall distribution in UAE. The influence of humid zone allows species presence in larger number and around all the year.

ACKNOWLEDGEMENTS

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Odonata checklist of Nature Reserve and SAC (Special Area of Conservation) "Vallone di Piano della Corte" (Sicily, Italy)

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ABSTRACT   From March to September 2018 and 2019, a first monitoring of Odonata promoted by CUTGANA was conducted inside a site of Natura 2000 network, named "Vallone di Piano della Corte", near Agira (Enna), Sicily. A total of 21 different species were recorded, including *Pyrrhosoma nymphula* (Sulzer, 1776), for which there are no stations reported for this side of Sicily.

KEY WORDS   Dragonflies; Erei; Natura 2000; Odonata; *Pyrrhosoma nymphula*.

INTRODUCTION

Up to date, 165 species of Odonata are so far reported for the Mediterranean basin area (Boudot et al., 2009; Boudot & Kalkman, 2015). Among them, 144 are reported for Europe and 96 for Italy, of which 58 species occurring in Sicily (Riservato et al., 2014; Galasso et al., 2016; Viganò et al., 2017; Surdo, 2017; Corso & Penna, 2020). The number of species in Sicily is probably destined to grow further thanks to the intensification of the knowledges and research activities, until today mostly limited to main wetlands and water habitats.

This short note is a small contribute to the information on Sicilian dragonflies related to a never before studied area for what concerns this *taxon*.

MATERIAL AND METHODS

Study area

The study area, named "Vallone di Piano della Corte", is a valley crossed by a stream located in the middle part of the Erei mounts, Sicily, in the municipality of Agira, province of Enna (37°38'44.32"N 14°29'44.58"E, Fig. 1). This survey was primarily focused along the main stream which flows through to the valley, named "Torrente Brace", its tributaries, and all the surrounding minor water habitats such as small lakes, troughs and natural ponds (Fig. 2). The stream, starting from its source is up to 6 km long and has an extended surface of 146 hectares. In addition, the stream is included in the regional nature reserve "Vallone di Piano della Corte".

The nature reserve was established from Regione Sicilia (Assessorato Regionale del Territorio e dell'Ambiente) in 2000, and is presently managed by CUTGANA (Centro Universitario per la Tutela e la Gestione degli Ambienti Naturali e degli Agro-ecosistemi) of Catania's University. Furthermore, since 2005, 450 hectares of the valley are included in the network of Natura 2000 as Special Area of Conservation (SAC ITA060007). The valley of Brace's stream is bordered by reliefs

of 500–800 m a.s.l., including mount "Teja", where the town of Agira is located. The early stretch of the stream, located in "Santa Venera" disctrict, crosses sandy soils and is characterized by a luxuriant vegetation with forest of *Salix alba* and *Populus alba*, with some old trees of *Populus nigra* (Fig. 3). The remaining part of the stream, inside the so-called district "Ponte", is instead characterized by clay soils and trees of *Tamarix* sp. alternated to open meadows. The whole stream bed is usually dry for the most of the year, with the occasional exception of residual pools which are instead always present in the middle due to spring waters, even during the Summer. For additional botanic insights see Costanzo et al. (2005). In the study area, near to "Monte S. Agata", there are also some drinking troughs for cattle and two artificial water reservoirs, about 5 and 1,300 m² wide, respectively.

Additional data about Odonata were also obtained in the nearby area of "Monte Chiapparo", included in another Special Area of Conservation Natura 2000 (ITA060014) as well in an artificial water reservoir 700 m² wide. The site is located north of mount Chiapparo, in the Contrada San Nicola.

Samples

The method used has been the "capture-identification-release", walking through transects along the main stream of the valley and from points of interest near to natural small lakes, ponds and drinking troughs. They were carried out once a week, from March 2018 to September 2018 and from March 2019 to September 2019. The sampling was done always during the morning and with good weather conditions (no rain and no wind), when the dragonflies activity is more intense and the identification was made mainly collecting and releasing the specimens with entomological nets, or, in other cases, through direct observation with binocular and collection of photographic material.

RESULTS

Systematics

Ordo ODONATA Fabricius, 1793
Subordo ZYGOPTERA de Sélys Longchamps, 1854
Familia CALOPTERYGIDAE Sélys, 1850

Genus *Calopteryx* Leach, 1815

Calopteryx haemorrhoidalis (Vander Linden, 1825)

This species has been regularly observed in 2018/19, from early May to September, even without a high numbers of imagoes, especially in the middle trait of the Brace stream, mostly shady and rich in vegetation. Breeding was confirmed in the study area.

Familia LESTIDAE Calvert, 1901

Genus *Sympecma* Burmeister, 1839

Sympecma fusca (Vander Linden, 1820)

Imagoes evenly observed in the whole area from April to September of both years, almost in all the habitats along the Brace stream and in the troughs. Breeding confirmed.

Genus *Chalcolestes* Kennedy, 1920

Figure 1. Geographical location of the study area, in the centre-north Sicily, west side of mount Etna.
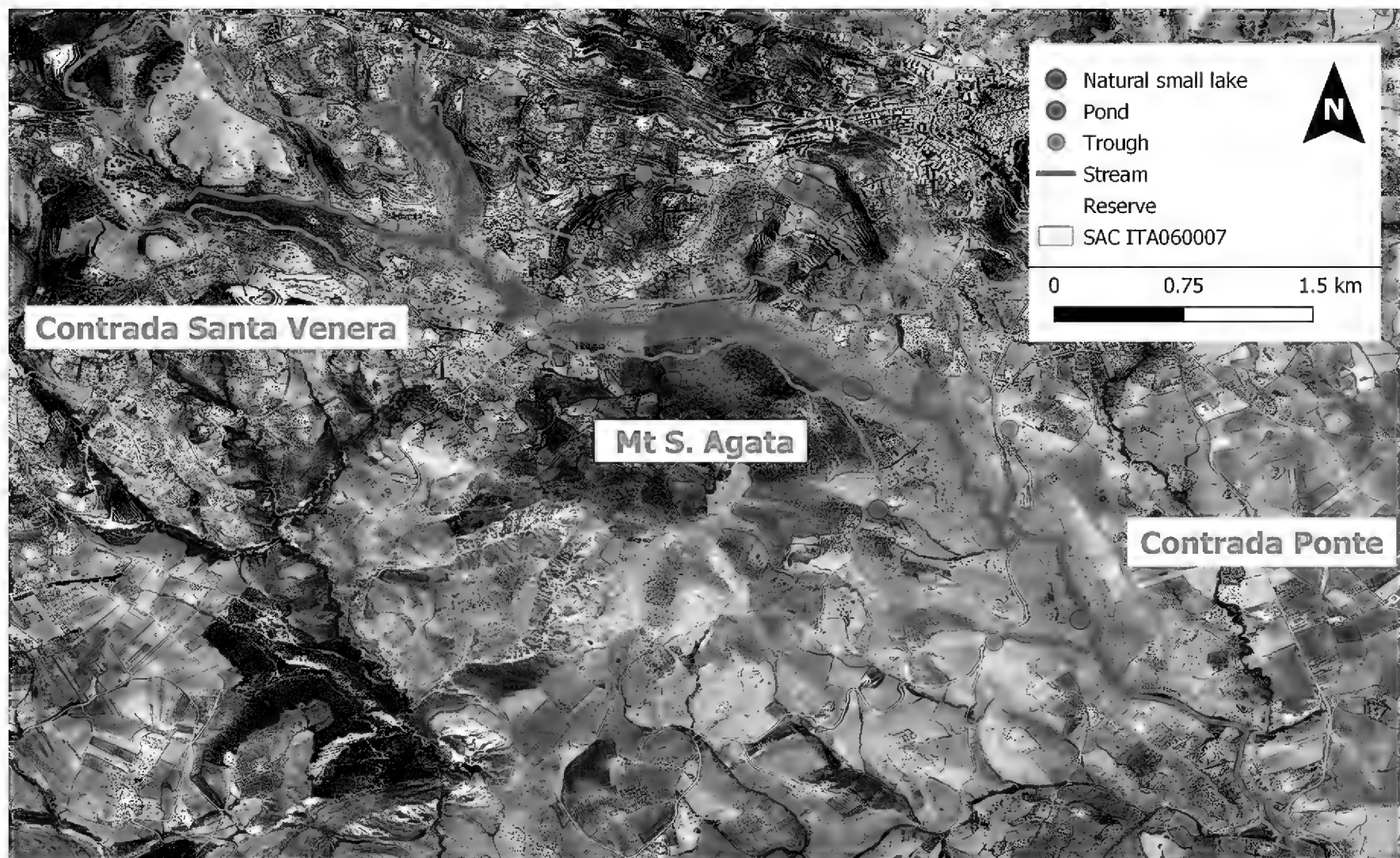
Figure 2. Map of Vallone di Piano della Corte, with the main points of interest for Odonata monitoring used for this survey and delimitation of protected areas.

Figure 3. Representative habitat with *Salix alba* trees along side the Brace stream, "Vallone di Piano della Corte", near Agira (Enna), Sicily.

Chalcolestes viridis (Vander Linden, 1825)

Males and females imagoes observed regularly from June to September of both years but only in some shady stretches of the upper and middle part of Brace stream. Found also at a great distance from water in a coniferous wood in September 2019. Breeding confirmed.

Familia COENAGRIONIDAE Kirby, 1890

Genus *Ceriagrion* Sèlys, 1876

Ceriagrion tenellum (de Villers, 1789)

Regularly observed from May to September of both years mainly in open habitats as ponds, troughs and in the small lake, but also in some opened and sunny stretches of Brace stream. Breeding was confirmed.

Genus *Coenagrion* Kirby, 1890

Coenagrion scitulum (Rambur, 1842)

Pretty common species, imagoes observed regularly from May to September 2018/19, mainly in open habitats as ponds and in the small lake, but also in some opened and sunny stretches of Brace stream. Breeding confirmed.

Genus *Erythromma* Charpentier, 1840

Erythromma viridulum (Charpentier, 1840)

Few imagoes, males and females, observed from June to late September, both in 2018 and 2019, exclusively in ponds and small lake. Breeding was confirmed for the study area. Also recorded in Monte Chiapparo.

Genus *Ischnura* Charpentier, 1840

Ischnura genei (Rambur, 1842)

The commonest damselflies in the area, regularly observed from March to September in all the habitats monitored. Breeding was confirmed. Also recorded in Monte Chiapparo.

Genus *Pyrrhosoma* Charpentier, 1840

Pyrrhosoma nymphula (Sulzer, 1776)

Few imagoes observed (4–5), also during mating and oviposition (Fig. 4), on 28.V.2018. This species was found only in one station, in the upper part of the study area, named Contrada Santa Venera.

Subordo ANISOPTERA de Sélys Longchamps, 1854
Familia AESHNIDAE Rambur, 1842

Genus *Aeshna* Fabricius, 1775

Aeshna isoceles (Müller, 1767)

Only 1 imago observed in flight on 28.V.2018, along the Brace stream near S. Agata mountain.

Aeshna mixta (Latreille, 1805)

Only 3 imagoes, 2 males and 1 female, observed in September 2018. These low records are probably linked to the period of this study, since the species is usually seen starting to September to November (Galliani et al., 2017). Breeding was not confirmed in the study area.

Genus *Anax* Leach, 1815

Anax imperator (Leach, 1815)

Regularly observed from June to September 2018 and 2019 in all the sunny and open habitats (not along the Brace stream), both sexes, also during mating and oviposition. Also recorded in Monte Chiapparo.

Anax parthenope (Selys, 1839)

Only few imagoes observed in August and September 2018 and 2019, exclusively near the pond of mountain S. Agata. Breeding highly probable but not confirmed. Also recorded in Monte Chiapparo.

Familia LIBELLULIDAE Rambur, 1842

Figure 4. *Pyrrhosoma nymphula* oviposition photographed on 28.V.2018 (photo P. Galasso).

Genus *Orthetrum* Newman, 1833

Orthetrum brunneum (Fonscolombe, 1837)

The commonest *Orthetrum* in the study area: males regularly observed in all the sunny habitats from to May to September, both in 2018 and 2019. Also observed in tandem and mating.

Orthetrum coerulescens (Fabricius, 1798)

Only few males observed in August 2018 in the middle part of Brace stream, in small natural lakes. Breeding was not confirmed.

Orthetrum trinacria (Selys, 1841)

Few males and females regularly observed in August and September of both years but exclusively in the big pond/lake of S. Agata mountain. Breeding probable but not confirmed.

Genus *Crocothemis* Brauer, 1868

Crocothemis erythraea (Brullé, 1832)

Commonly observed in all kinds of habitats, from April to September, even without high numbers of imagoes, also in tandem, mating and oviposition. Also recorded in Monte Chiapparo.

Genus *Sympetrum* Newman, 1833

Sympetrum fonscolombii (Selys, 1840)

Observed from late March to September 2018 and 2019 in sunny and open habitats (not along the Brace stream), often far away from water, also in tandem and mating. Also recorded in Monte Chiapparo.

Sympetrum meridionale (Selys, 1841)

Only few imagoes (4–5 in total) observed in August and September 2018 and 2019 in sunny and open habitats (not along the Brace stream), also in tandem and mating.

Sympetrum striolatum (Charpentier, 1840)

The commonest *Sympetrum* in the study area; regularly observed from July to September of both years, exclusively in sunny habitats often far away from water, also in tandem and mating. Also recorded in Monte Chiapparo.

Genus *Trithemis* Brauer, 1868

Trithemis annulata (Palisot de Beauvais, 1807)

Few imagoes observed in August and September 2018 and 2019, exclusively near the pond of S. Agata mountain. Males and females also observed in tandem and a female during oviposition. Recorded also in Monte Chiapparo.

Genus *Selysiothemis* Ris, 1897

Selysiothemis nigra (Vander Linden, 1825)

ORDER	SUBORDER	FAMILY	SPECIES
Odonata	Zygoptera	Calopterygidae	*Calopteryx haemorrhoidalis* (Vander Linden, 1825)
		Lestidae	*Sympecma fusca* (Vander Linden, 1820)
			Chalcolestes viridis (Vander Linden, 1825)
		Coenagrionidae	*Ceriagrion tenellum* (de Villers, 1789)
			Coenagrion scitulum (Rambur, 1842)
			Erythromma viridulum (Charpentier, 1840)
			Ischnura genei (Rambur, 1842)
			Pyrrhosoma nymphula (Sulzer, 1776)
		Sub-total of families: 3	**Sub-total of species: 8**
		Aeshnidae	*Aeshna isoceles* (Müller, 1767)
			Aeshna mixta Latreille, 1805
			Anax imperator Leach, 1815
			Anax parthenope (Selys, 1839)
		Libellulidae	*Orthetrum brunneum* (Fonscolombe, 1837)
			Orthetrum coerulescens (Fabricius, 1798)
			Orthetrum trinacria (Selys, 1841)
			Crocothemis erythraea (Brullè, 1832)
			Sympetrum fonscolombii (Selys, 1840)
			Sympetrum meridionale (Selys, 1841)
			Sympetrum striolatum (Charpentier, 1840)
			Trithemis annulata (Palisot de Beauvois, 1807)
			Selysiothemis nigra (Vander Linden, 1825)
		Sub-total of families: 2	**Sub-total of species: 13**
	Total	**Families: 5**	**Number of species: 21**

Table 1. List of all the species recorded in the studied area of "Vallone di Piano della Corte", near Agira (Enna), Sicily.

Only few males observed from June to August 2018 and 2019, exclusively around the pond of S.Agata mountain. Breeding was not confirmed in the study area.

DISCUSSION AND CONCLUSIONS

A total of 21 different species were found in Vallone di Piano della Corte of which 8 belong to the Zygoptera suborder and 13 to the Anisoptera suborder. The following families are so represented: 1 species of Calopterygidae (Odonata, Zygoptera), 2 species of Lestidae (Odonata, Zygoptera), 5 species of Coenagrionidae (Odonata, Zygoptera), 4 species of Aeshnidae (Odonata, Anisoptera), 9 species of Libellulidae (Odonata, Anisoptera). In addition to these species, *Lestes barbarus* (Fabricius, 1798) was found exclusively in the nearby area of "Monte Chiapparo" and observed and photographed also in copulation.

Particularly interesting is the record of *Pyrrhosoma nymphula*, species found regularly in Sicily only on Nebrodi Mountains since 2009 up to 2019, and, occasionally in some stations on Hyblaean Mountains, Sicani Mountains and Madonie Mountains (Corso, pers. obs.). From a point of view of conservation, all the species recorded are currently included by the Italian Dragonflies Red List into the LC (Least Concern) IUCN category, both for the Italian and global population (Riservato et al., 2014b).

Despite the fact that Vallone di Piano della Corte is not a wetland, the diversity and heterogeneity of its habitats, including small artificial niches as troughs and water reservoirs, allowed to find up to the 37% of the Sicilian species in such a small area. The richness of species in the site encourage activities of ecoturism such as Dragonflies-watching.

This survey shows, once again, the importance of smaller water habitats, that for their essential ecological function, can be much richer of dragonflies and biodiversity in general than what we could have expected, mainly when water sources around are not available.

Considering the current deficiency of published data about dragonflies distribution in Sicily, this survey and the check list hereafter add important information to our fragmented knowledge, mainly for the central Sicily.

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Role of hymenopterous parasits Aphelinidae in the regulation of levels of *Lepidosaphes beckii* (Newton, 1869) (Homoptera Diaspididae) populations on orange trees in Rouiba area (Algeria)

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ABSTRACT   This present study put in evidence the dynamics of the populations of *Lepidosaphes beckii* (Newton, 1869) (Homoptera Diaspididae), and the study of the impact of its hymenopterous parasites *Aphytis lepidosaphes* Compere, 1955, *Aphytis chrysomphali* Mercet, 1912, *Aphytis proclia* Walker, 1839 and *Aspidiotiphagus citrinus* (Craw, 1831) on orange tree in the area of Rouiba. This diaspine develops three generations: one in autumn-winter, one in spring and one in summer. This scale insect presents a very marked affinity in the middle of the tree and the inferior faces of the leaves where the suitable conditions for its development seems to be offered. The natural mortality of *L. beckii* is of two types: climatic for young stages and physiological for the adult females. The parasitic incidence is almost the same for the young stages and the adult with respectively 22.24% and 23.89%. The global rate of parasitism is 23.24% insufficient to control its host.

INTRODUCTION

The cultivation of citrus fruits is of strategic importance for Algeria where it is a source of fruit supply and opportunities in the international market for citrus products. Among the Citruses, the Orange tree occupies the first place with 69.73%, then the Lemon tree with 6.94%, the Mandarin with 4.73% and the clementine with 1.82%. The citrus orchards are subjected to very high-pressure by several pests, in particular by the cochineals that appear to be the second pest after the Mediterranean fruit fly (Benassy & Soria, 1964). The diaspines represent 42.37% of all the cochineals and the genus *Lepidosaphes* Shimer, 1868 (Homoptera Diaspididae) is the most dominant with 14% (Belguendouz, 2005). While the cochineals in general, and *Lepidosaphes beckii* (Newton, 1869) in particular, cause important damages on citrus fruits, there are, however, predators and pests very poorly known in Algerian conditions. Our work

presents a contribution to the understanding of the ecology of *L. beckii* on Orange trees in the region of Mitidja, and its relationship with the parasites Hymenopter.

MATERIAL AND METHODS

Our study was carried out on a private farm in Rouiba (36°44'00''N and 3°17'00''E), located to the east of the Mitidja, 25 km from the capital Algiers and 7 km from the Mediterranean Sea. It is delimited to the north by the town of Ain-Taya, to the south by the town of Khemis El Khechna, to the east by the town of Reghaia and to the west by the town of Dar El Beida, over a period from September 2016 to July 2017. The plot is 2 hectares, divided into 9 blocks of 25 trees. Prospecting was done once per 10 days, with a sampling of 2 trees from each block on which we took 1 branch of 20 cm long and 2 leaves (in each cardinal direction as well as in the center of the tree). The collected plant material were brought back to the laboratory where we counted for each stage the total live, dead and parasitized individuals of *Lepidosaphes beckii* in order to assess the condition of the insect infestation and the evolution of its pests in according to the method of Vasseur & Schvester (1957).

Identification technique for identified pests

The identification of Aphelinidae is based on the study of their morphological characters (Ferrière, 1965). The technique consists in organizing the parasites harvested under a binocular loupe between blades and lamellae. The latter fixed by Faure's liquid. In our study, 4 species of parasites were identified on the populations of *L. beckii*: *Aphytis lepidosaphes, Aphytis chrysomphali, Aphytis proclia*, which are ectoparasites, and *Aspidiotiphagus citrinus*, which is an endoparasite.

RESULTS AND DISCUSSION

Biology of the insect scale

The analysis of the results reported in figure 1 a cross-breeding between larval and adult elements,

which is dictated by the natural life cycle of *L.beckii* where the percentages of these two populations are almost equal until mid-December when the larvae are present with 31.63% of the total number of individuals. Then there is a further progression in mid-January that reaches 76.30%. These are likely from the adult females during autumn. Thereafter, the larval populations will undergo gradual regression and reach their lowest rate by the end of April. A further increase in staffing is then noted for the highest rate in July (81.59%). For adult fluctuations, the adults displayed their highest percentage (68.37%) only at the beginning of December. These females are likely the cause of the larvae observed during the month of November. Thereafter, these adult forms display a second peak (75.33%) in the first then days of May (Fig. 1).

The results indicate an overlap of three annual generations in *L. beckii*: spring, summer and autumn generation. Our results confirm those reported by Biche et al. (2011), Biche et al. (2012), Adda (2010) in the same region of the Orange tree. On the other hand, Meghazi (2010) found 2 generations: fall and spring on the Orange tree in Rouiba. Benassy et al. (1975) reported that the Cochineal develops 2 generations per year in France, 3 in Tunisia, 4 in Naples (Italy), Egypt and Morocco. In Chile, Zuniga (1971) reported three annual generations in the northern region, two to three generations in the central region and only one generation in the southern region. On the other hand, in Eastern Cape in South Africa, De Villiers (1998) reported four annual generations.

The overall study of *L.beckii* shows that the central orientation is affected the most by this
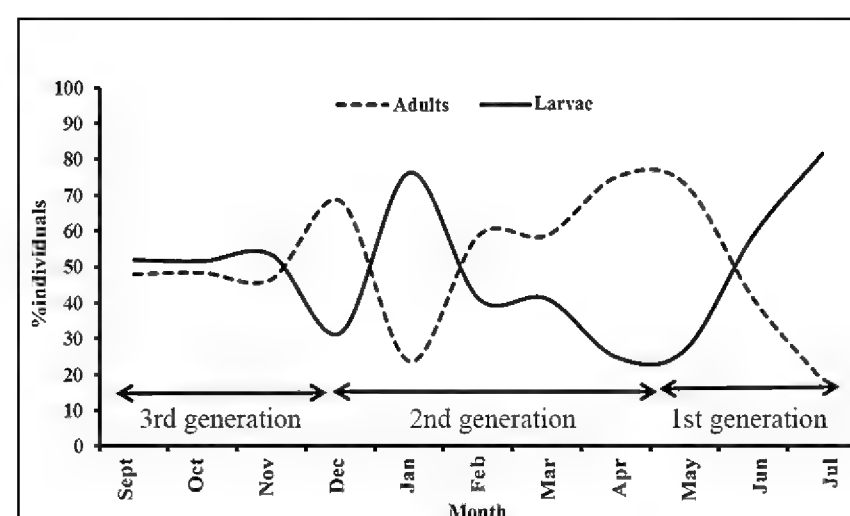

Figure 1. Fluctuation of larval and adult populations of *Lepidosaphes beckii*.

cochineal with 38.36%, followed by the eastern orientation with 22.12% of the total population (Fig. 2). Our results coincide with those obtained by Gherbi (2010) and Meghazi (2010) who worked in the same region. On the other hand, on the clementine trees, Mouas (1987), who worked in Annaba, and Khoudour (1988) in the Chebli region, report that *L. beckii* is found in the northern orientation. The vegetal organ has a very remarkable influence on the abundance of this cochineal, where the leaves contain 91.90% of the total population, mostly on the lower surface (56.31%)than on the upper surface (35.59%) (Fig. 2). This situation provides the cochineal with favorable fixation conditions. Indeed, the leaf blade has a very fine texture, favouring the bites of opophages insects. Our results are consistent with those found by Gherbi (2010). On the other hand, Mouas (1987), Khoudour (1988), Adda (2006) and Adda (2010) note that the highest rate of the living population of *L. beckii* is located in the upper surface of the leaves.

Study of mortality

In view of the results shown in the figure below, we note that overall mortality is significantly higher in larval populations (43.87%) than in adult populations (30.86%). Nevertheless, considerable fluctuations have been recorded throughout the period of study, stretching from September to July (Fig. 3). This mortality is likely related to the structure of the larval shield and the climatic conditions of the winter season where the minimum temperatures displayed 6.2°C in December, 2.3 °C in February and 7.6 °C in March. Instead, the strong rainfall recorded during the month of May was 75 mm, compared to 18 mm in April and 2.55 mm in June. Regarding adult mortality, it is mostly physiological. Indeed, the females after oviposition, die immediately. Mouas (1987), Meghazi (2010), and Gharbi (2010) found that overall adult mortality is quite significant in larvae.

The recorded results show that the overall mortality rate remains very high where the abundance of the cochineal is very pronounced. Nevertheless, the highest mortality rates were recorded at the central and southern orientation of the tree, with 19.78% and 16.84%, respectively, while on leaves is 52.72% (52.72%) (Fig. 4). This
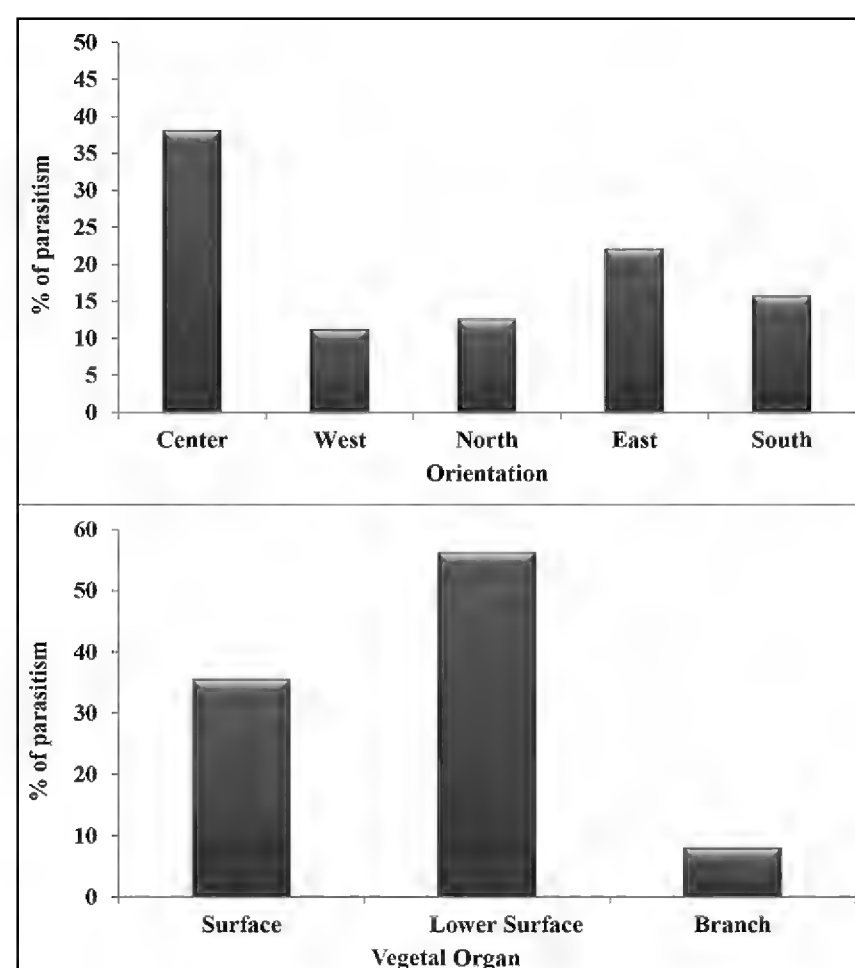

Figure 2. Spatiotemporal distribution of the overall population of *Lepidosaphes beckii*
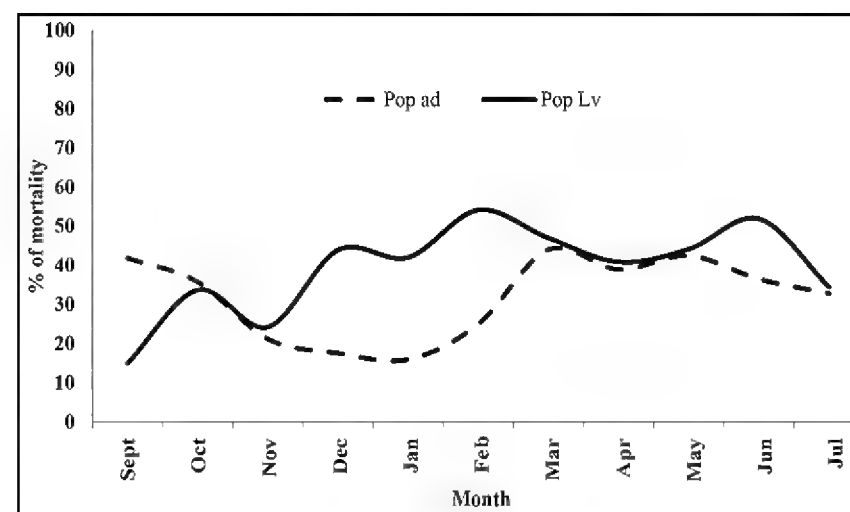

Figure 3. Mortality of *Lepidosaphes beckii* larvae and adults on the orange tree.

result is probably related to the exposure of these leaves to the different climatic hazards during the winter or summer period, namely high temperatures, winds, and hail, as well as the chemical treatments carried out in the orchard.

Inventory of cochineal pests listed on Orange tree at Rouiba

Periodic and random sampling of parasite populations remains an efficient method that allows, in addition to the inventory, to clarify

certain bioecological parameters, on the one hand, and, on the other hand, to assess possibly the entomological role of each species identified for their biological control jobs. The parasite inventory of *L. beckii* highlights 4 species of Hymenoptera Aphelinidae represented by *Aphytis lepidosaphes* Compere, 1955, *Aphytis chrysomphali* Mercet, 1912, *Aphytis proclia* Walker, 1839 and *Aspidiotiphagus citrinus* (Craw, 1831) (Table 1).

Aphytis lepidosaphes is an ectoparasite that develops at the expense of females adult and 2nd stage male larvae.

Aphytis chrysomphali, *Aphytis proclia* are ectoparasites that develop at the expense of 2nd stage male larvae.

Aspidiotiphagus citrinus is an endoparasite that develops at the expense of the larvae of 2nd stage larvae of *P. ziziphi* and of young females (Ferrière, 1965). We found it for the first time on female larvae in Algeria.

Study of the Parasite incidence

The results recorded in the graph below show that the overall rate of all-stage parasitism in *L.beckii* fluctuates throughout the study period. This fluctuation first passes through a first peak during November with a rate of 30.49% and the second peak in April with a rate of 20.64% and reached the highest rate of parasitism during the month of July with 67.62% (Fig. 5). Overall, the parasitism recorded in the populations of *L.beckii* is 23.24%. This rate is similar to that reported by Meghazi (2010) who worked in the same region of the Orange tree (20.45%). On the other hand, compared to the work of Adda (2006), the parasitic incidence was higher, as the author noted 4.01% adult parasitism. This is likely due to the declining chemical treatments carried out in the study orchard.

The fluctuation of parasitism goes through two main periods: autumn-winter and spring-summer. During the first period, rates varied between 53.81% (September) and 25.58% (December), due to the availability of host larvae for parasites during this period. Thereafter, Parasitism parasitism considerably decreases to vary between 13.78% (January) and 11.03% (February). This decrease is likely related to pesticide application in the study orchard. There was not parasitic activity in the
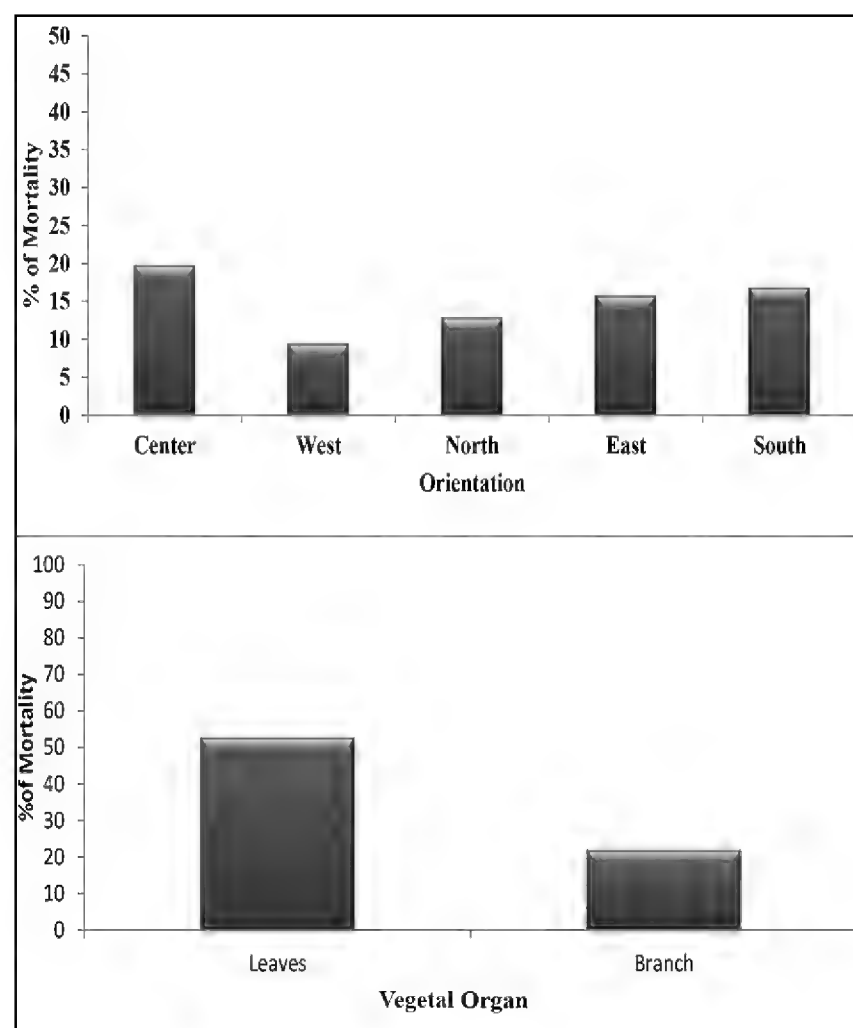

Figure 4. Spatiotomporel mortality of de *Lepidosaphes beckii*.

Class	Order	Family	Species	
Insecta	Hymenoptera	Aphelinidae	*Aphytis lepidosaphes*	Ectoparasites
			Aphytis chrysomphali	
			Aphytis proclia	
			Aspidiotiphagus citrinus	Endoparasite

Table 1. Description of the parasites of *Lepidosaphes beckii* on the orange tree in the region of Rouiba.
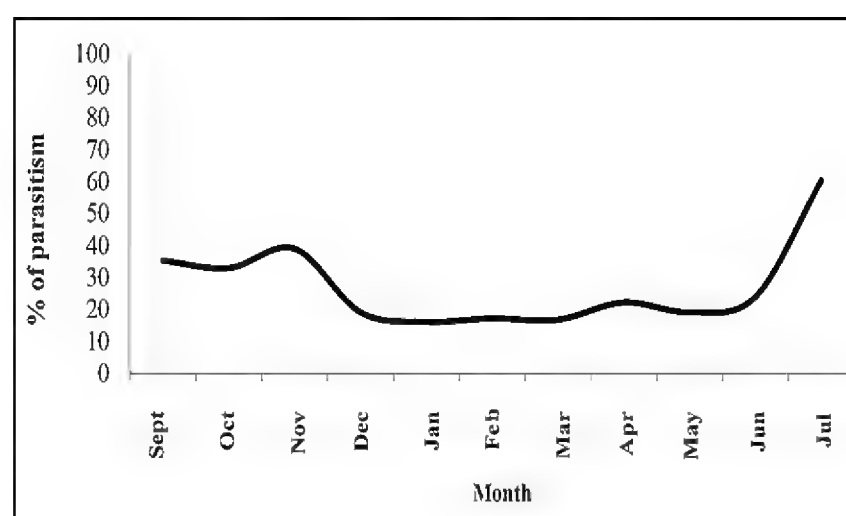

Figure 5. Overall Parasite Incidence of *Lepidosaphes beckii* populations

larval populations until the end of March. In fact, the parasitism of this second period varies between 24.94% (April) and 28.65% (July) (Fig. 6).

Due to the availability of agreater presence of adult cochineal stages, and in contrast to larval populations, the parasite incidence in adults noted for 3 periods: autumn-winter, spring and summer. The first peak of the parasite incidence was recorded in November with 30.49%, the second in April with 21.72% and the third in July with 67.62% (Fig. 7).

Based on the results obtained, it appears that the orientation and the vegetal organ do not have a significant effect on the distribution of the parasite on the tree. Abundance of host cochineals would result in a synchronous increase in the numbers of the parasite populations (Fabres, 1979). Nevertheless, the highest parasitism were recorded in the, east and South, orientations, with 28.86% and 28.75%, respectively (Fig. 8). It should be noted that the lowest rate recorded was in the population in the central orientation of the trees. Pests seem to avoid shady areas of the tree and prefer to look for cochineal fixed in sunny places. Our results are similar to those reported by Adda (2006) and Meghazi (2010) , whose northern orientation is the most preferred by parasitism.

For the spatial distribution of pests, it should be noted that the latter are more active on the upper surface (27.16%) than on the lower surface of the leaves (21.47%) and twigs (18.24%). These places seem to explain the requirement of parasites in micro-climatic conditions since in these places the sunshine is better.

The host's females perforated shields correspond to the release of adult parasites and reveal the presence of parasitoids. During the study period, we found the presence of a very large number of these females shields and puparia holes. However, they display three peaks, including 217 females shields and puparia holes in the month of October, then 509 in March and lastly in June with 436 females shields and puparia holes (Table 2).

Comparative global parasitism rate

Based on the results recorded in figure 9, it is apparent that the parasitoids attack much more the female stages of the cochineal, in wich 23.89% of the adults are parasitized. On the contrary, the males are attacked throughout their life cycle, which provides the parasitoid with nutritional conditions for better development. We also noted that parasitism remains constant in the female population throughout the study period with fluctuations in three peaks; the first in November (30.70%), the second in April (21.7%) and the third in July (67.90%) (Fig. 9). A similar work in Egypt, on the incidence of external parasites of *L. beckii*, has shown that rates of parasitism are not very high. In fact, Hafez et al. (1987) reported rates of parasitism between 29.7 and 40.2% in October-December, 21.6% in January, 16,1% in May and 15.4% in June.

The rate of parasitism of the males remains low compared to that of females with 19.11%. Probably, the male stages do not offer the optimal nutritional conditions for better development of the parasitoid because they no longer feed during the nymph stages to become adult (Fig. 10).

The rate of parasitism in 2nd stage male larvae remained low at 7.42% compared with lin 2nd stage
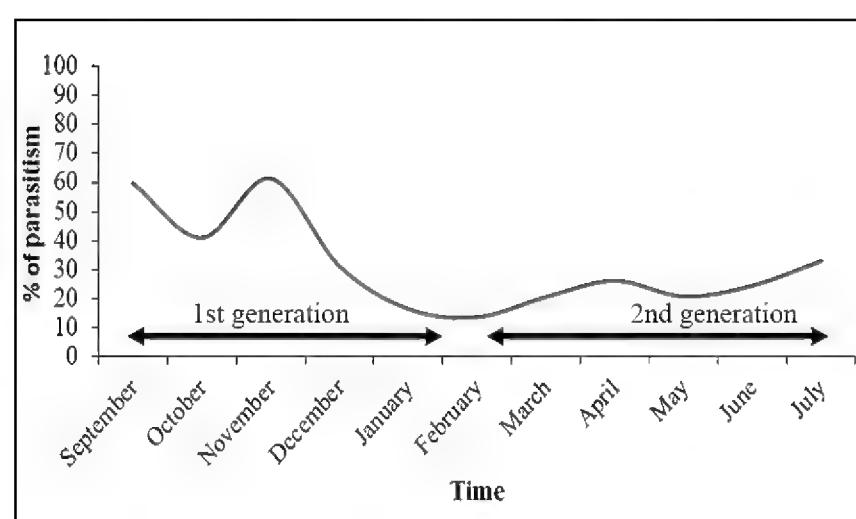

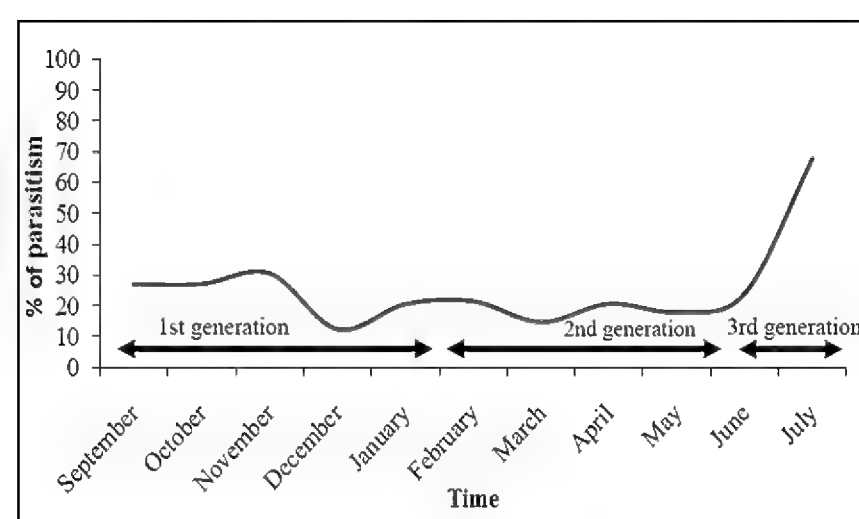Figure 6. Parasite impact of larval populations of *Lepidosaphes beckii*

Figure 7. Parasite incidence of adult populations of *Lepidosaphes beckii*

female larvae. Presumably, these male larvae do not offer optimal nutritional conditions for better development of parasitoid. Three peaks of parasitism have been observed: the first in October (43.18%), the second in March (18.42) and the last in June (8.33%) (Fig. 11).

Based on the results recorded in figure 12, it is apparent that parasitoids attack much more the female larval stages of the cochineal (24.82%).
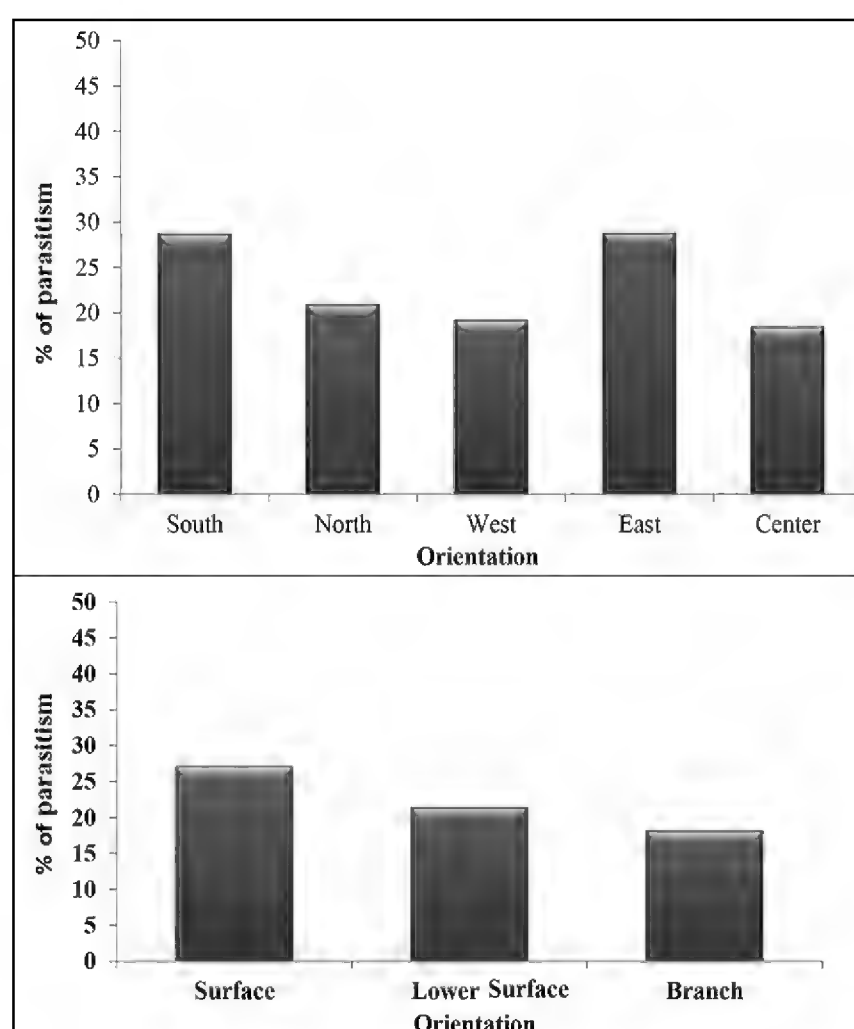

Figure 8. Parasite incidence of *Lepidosaphes beckii* populations according to the orientations and vegetal organs.

Date	Shields holes	Puparias holes	(S+P) Holes
September	70	2	72
October	196	21	**217**
November	134	11	145
December	103	12	115
January	112	15	127
February	163	8	171
March	495	6	**501**
April	333	3	336
May	242	6	248
June	435	1	**436**
July	290	10	300
Total	2573	95	**2668**

Table 2. Parasitic incidence of parasites in *Lepidosaphes beckii* populations by taking into account holes puparias and females shields.

Given the availability of these larvae throughout our sampling, parasitoids provide the nutritional conditions for better development. We also noted that parasitism in these larvae goes through three peaks: the first during the month of September (59.60%), the second during the month of November (61.32%) were the highest rate and the third were recorded during the month of July (32.82%) (Fig. 12).

CONCLUSIONS

The study allowed us to undertake a new relative approach to the role of parasites Hymenoptera Aphelinidae in limiting populations of *L. beckii*. However, the study of the population dynamics of the pest is a key element in understanding the biological phenomena that govern the evolution of the pest during the seasons and to better define the techniques of control to adopt.

Based on the results obtained, we conclude that there is an overlap of three generations in year of *L. beckii*: one in autumn-winter, one in spring, and one in summer. The spatial distribution of this species on the tree is closely related to the microclimate created within the tree from which the species has a marked preference for its central orientation. This place seems to give it the favorable conditions for its optimum development.

The incidence of natural enemies is the result of the action of Hymenoptera parasites of the genus *Aphytis* Howard, 1900 and *Encarsia* Foerster, 1878, whose activity is mainly noticeable in full and spring. Three ectoparasites Hymenoptera species have been identified in the populations of *L. beckii*, *A. lepidosaphes, A. chrysomphali*, and *A. proclia*, and a single endoparasite species, *Aspidiotiphagus citrinus*, during our study. It should be noted that this is the first time in Algeria that the presence of these parasitoids on the larval forms of the cochineal is observed. All of the studies conducted so far on the Diaspines have never reported this type of information.

We noted that *A. lepidosaphes* is present only in adult males and females. However, they are much more present on the females of the cochineal as it offers optimal nutritional conditions. The overall rate of parasitism approximates 23.89%. Two
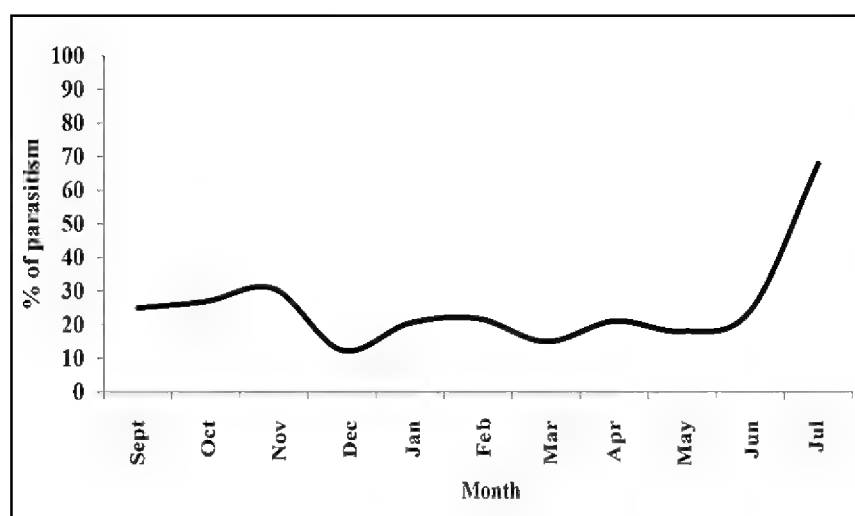
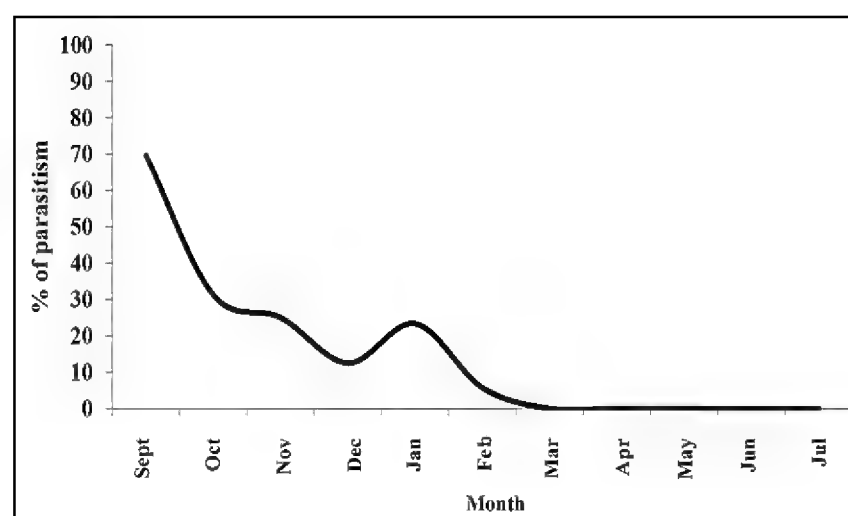Figure 9. Parasite incidence of *A. lepidosaphes* on females *Lepidosaphes beckii.*

Figure 10. Parasite incidence of *A. lepidosaphes* on the males of *Lepidosaphes beckii.*
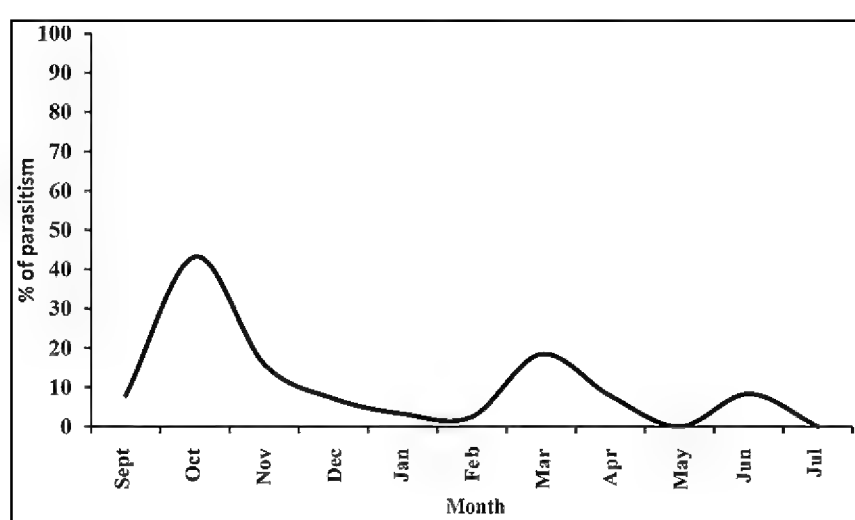

Figure 11. Parasite incidence of *A. chrysomphali* and *A. proclia* on 2nd stage male larvae of *Lepidosaphes beckii.*
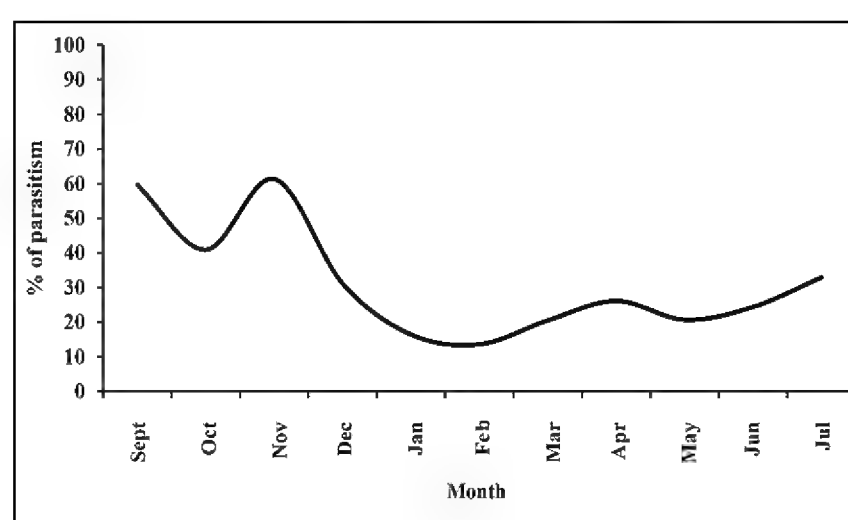

Figure 12. Parasite incidence of *A. citrinus* on 2nd stage female larvae of *Lepidosaphes beckii*

ectoparasites, *A. proclia* and *A. chrysomphali*, with a parasitism rate of 7.01%, and an endoparasite *A. citrinus*, were identified on 2nd stage male larvae. The latter is the only parasite that develops on 2nd stage female larvae with an overall rate of 22.19%.

Our work provides further information on the impact of parasitoids *A. lepidosaphes*, *A. chrysomphali, A. proclia* and *Aspidiotiphagus citrinus,* for a possible rational management of integrated control methods in good conditions.

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Growth and mortality of *Conger conger* (Linnaeus, 1758) (Pisces Congridae) in the Algerian basin

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ABSTRACT   The growth and mortality of *Conger conger* (Linnaeus, 1758) (Pisces Congridae) were determined from 295 individuals, ranging from 38.9 to 174 cm total length, collected in the Algerian basin. The von Bertalanffy growth equation was established on the basis of the analysis of size structures by the ELEFAN I method, and is written as follows: $L_t=224.4*(1-e^{-0.2t})$. The height-weight relationship shows a higher allometry $Wt=0.0003*Lt^{3.4}$. The total (Z) and natural (M) mortality rates are 1.28 and 0.25 per year respectively. The exploitation rate E = 80% seems to show an overexploitation of the *C. conger* stock in the Algerian basin, for which we propose for this situation, management scenarios for this fishery, based on changes in fishing effort and variations in the size of the mesh of the trawl.

KEY WORDS   Algerian basin; *Conger conger*; ELEFAN I; growth, mortality; overexploitation.

INTRODUCTION

The European conger *Conger conger* (Linnaeus 1758) (Pisces Congridae) occurs in the North-East Atlantic, from Iceland and Norway to Senegal, but also in the Mediterranean Sea and West of the Black Sea (Froese & Pauly, 2015).

This species has an elongated subcylindrical body (Fig. 1), compressed behind the anus. The profile of the head is depressed above the eye, which is small with a wide and flattened inter-orbital space. The muzzle is slightly prominent with an oral commissure in the posterior half of the eye. Both jaws are armed with an outer row of very compressed and tight incisiform teeth. The dorsal and anal fins meet the caudal, with segmented rays. The

dorsal fin begins above the tip of the pectoral fins (Fischer et al., 1987).

The European conger is a marine benthic species and is caught on rocky and sandy bottoms with a very wide bathymetric distribution, ranging from 40 m to 800 m deep (Dieuzeide et al., 1959; Fischer et al., 1987; Quéro & Vayne, 1997; Wood, 2011). They stay close to the coast when they are young and move to deeper waters as adults.

The European conger reaches sexual maturity at the age of 5–15 years (Fischer et al., 1987), and will reproduce in open water over the deep sea where each individual lays several thousand eggs (Quéro & Porché, 2005; Correia et al., 2011; Wood, 2011). It takes 2–3 years for the young people to return to the coastal habitat.

It is a very voracious carnivorous species. It feeds mainly at night, and its diet consists mainly of bottom-dwelling fish, crustaceans and cephalopods (Fischer et al., 1987).

The European conger eel is fished by beach seines, trawls, gillnets, bottom longlines, traps and handlines. The species is regularly present on Mediterranean markets (Fischer et al., 1987).

This species is a valuable species in the Algerian market, in which it is regularly present and is exploited both by commercial and recreational fishing, and caught by several fishing gears, mainly bottom trawls, hooks and longlines (Correia et al., 2011). Thus, it represents one of the species most caught by recreational fishing in Algeria (Sahi & Bouaicha, 2003). This seems to show a trend towards overexploitation of the stock. However, the

species is still not well known, and to our knowledge, no studies on the growth of *C. conger* in the Algerian basin have been published.

In this context, we are interested in the study of this species by estimating these growth and exploitation parameters.

MATERIAL AND METHODS

Data source

Sampling was carried out during the period from May 2014 to June 2015 from trips to the Algiers fish market, the ports of Bouharoun, Cherchell, Cap Djinet, Oran, Beni Saf, Mostaganem and the Fouka fishing shelter.

These outings made it possible to collect height and weight data from 295 individuals from *C. conger*, who come from the different regions of the Algerian basin (Fig. 2).

Study methods

Growth parameters were estimated from size frequency distributions using indirect numerical methods. Either by converting these distributions into age composition by graphical methods such as the Bhattacharya method (1967) or computerized
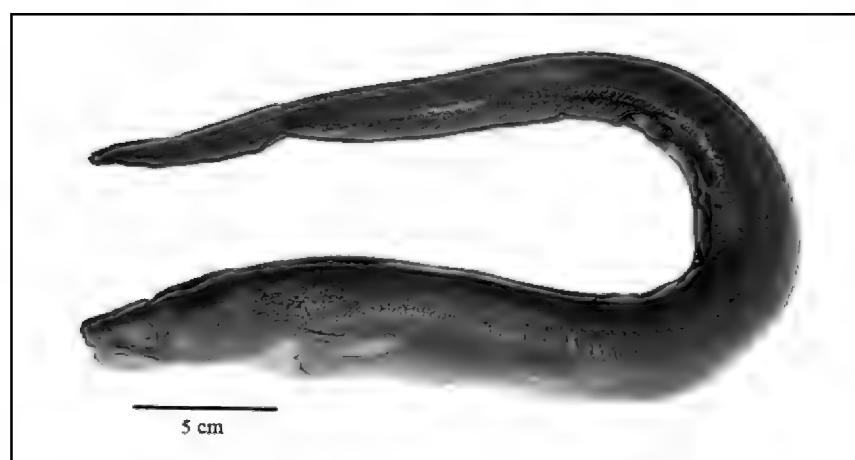

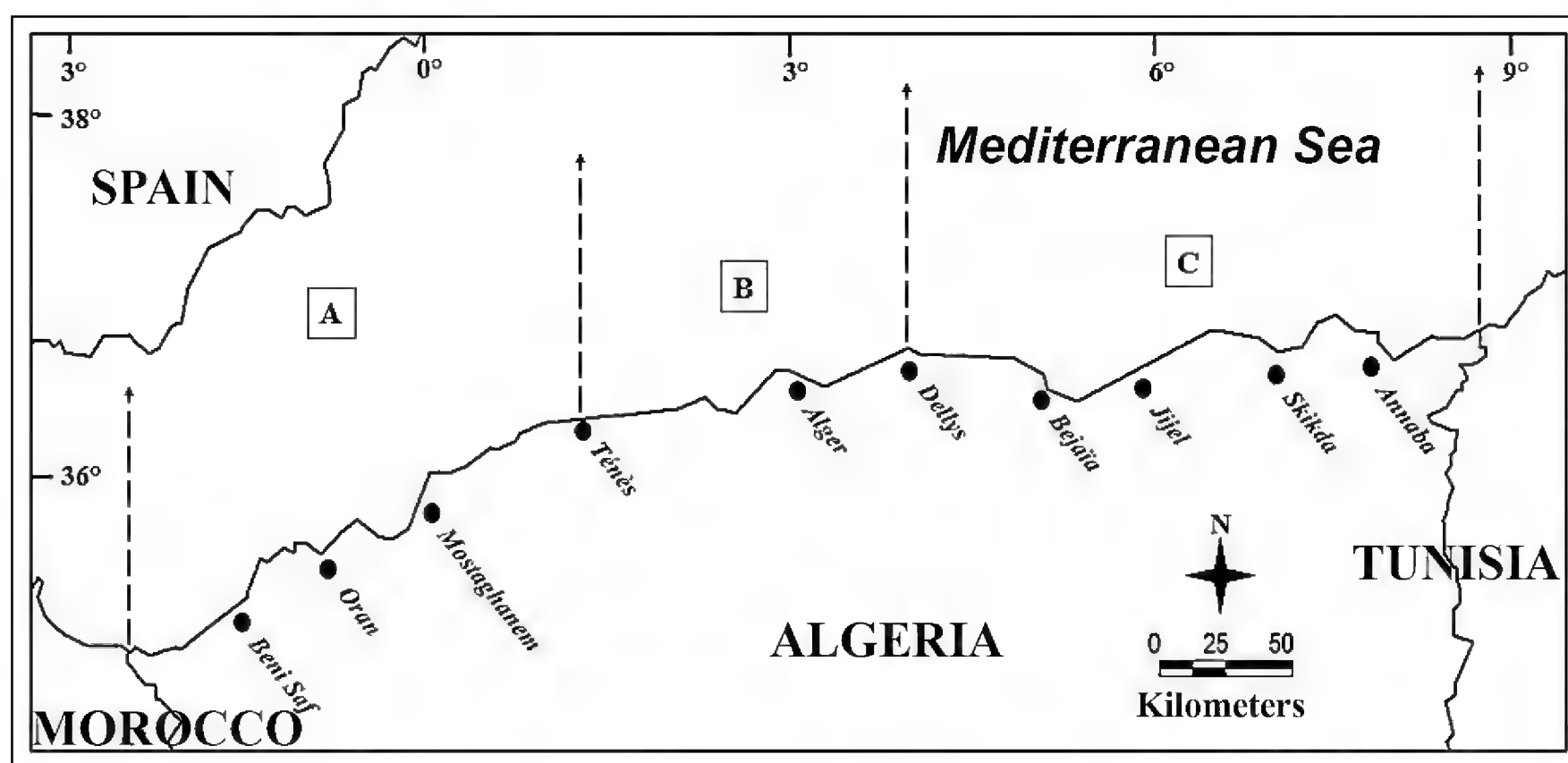Figure 1. *Conger conger* (Linnaeus, 1758)

Figure 2. Map of the Algerian Basin (A: Western Region, B: Central Region, C: Eastern Region).

methods such as the NORMSEP program or directly by analyzing these size structures using the ELEFAN I (Electronic Length Frequency Analysis) software (Sparre & Venema, 1996), which includes two main steps (Sparre & Venema, 1996; Pauly & Moreau, 1997): Step 1: restructuring the length frequencies; Step 2: adjusting a growth curve.

The age-length key obtained by the NORMSEP software is used to estimate growth parameters (L∞, K and t0) using FISAT II software using the Tomlinson & Abramson (1961; Hemida, 2005) method based on least square type adjustment. This method estimates the parameters in such a way that the sum of the squares of the differences between the model and the observations is minimal.

It should be noted that FISAT II software gives by default t0 = 0, for this purpose, this parameter is estimated by the Pauly equation (1983) using the formula:

$$Log_{10}(-t_0) = -0.3922 - 0.2752 * Log_{10}L_\infty - 1.038 * Log_{10}K$$

It is often useful to be able to accurately assess the weight of a fish knowing its size. To do this, there is a simple relationship that links length to mass, and is written as (Sparre & Venema, 1996):

$$W = a * L^b$$

W: body weight of the fish; L: total length; a and b are constants, of which b: represents an allometric coefficient, and when: b < 3: minor allometry; b = 3: isometry; b > 3: major allometry.

However, the type of allometry is confirmed by the t-test (Schwartz, 1992), based on the comparison of a calculated slope (P0 = b) with a theoretical slope (P = 3):

$$t = \frac{|P_0 - P|}{SP_0}$$

If t < 1.96: the difference is not significant; if t ≥ 1.96: the difference is significant.

The value of W_∞ is obtained from the equation:

$$W_\infty = a L_\infty^b$$

Where: a and b are those of the height-weight relationship.

The natural mortality rate M was estimated from the empirical equation of Djabali et al. (1994), which was established on the basis of the growth

and mortality parameters of 56 live fish stocks in the Mediterranean:

$$Log_{10} M = 0.0278 - 0.1172 Log_{10} L_\infty + 0.5092 Log_{10} K$$

Total mortality is estimated by Pauly (1984) method, using FISAT II software (Gayanilo et al., 2005), from size frequency distributions and growth parameters K and L∞. Knowing Z and M, F can be deduced from the equation: Z=M+F.

The analysis of the state of the stock can be carried out by calculating the exploitation rate (E) which provides information on the importance of fishing pressure using the following equation (Moreau et al., 1995):

$$E = \frac{F}{M + F}$$

When E is greater than 50%, this implies biological overexploitation.

RESULTS AND DISCUSSION

Growth parameters

The size data of 295 specimens of *C. conger*, vary between a minimum of 38.9 cm and a maximum of 174 cm. These data were grouped into a size frequency distribution table with a 10 cm step (Table 1).

The age-length key obtained by the Bhattacharya decomposition method was adjusted by the NORMSEP program (FISAT II), by successive iterations (Table 2, Fig. 3).

The age of the conger (*C. conger*) obtained from the decomposition of size frequency distributions varies between 1 and 3 years, which is not the case for previous studies. Correia et al. (2009) grouped the age of the European conger between 2 and 12 years from the reading of otoliths. The latter result is similar to those of previous studies (Sbaihi et al., 2001; Correia et al., 2009).

This difference is mainly due to the methods used to determine age and the study area where the sample is taken.

From the age-length key obtained, the growth rate between age group 1 and 2 is about 17.88 cm/year, and about 33.1 cm/year between age group 2 and 3. This is not in line with the von Bertalanffy growth curve since the growth rate be-

Classes (cm)	Classroom centres (cm)	Effective
38 − 48	43	66
48 − 58	53	73
58 − 68	63	62
68 − 78	73	40
78 − 88	83	21
88 − 98	93	13
98 − 108	103	6
108 − 118	113	5
118 − 128	123	3
128 − 138	133	3
138 − 148	143	1
148 − 158	153	1
158 − 168	163	0
168 − 178	173	1
	Σ	295

Table 1. Distribution of *Conger conger*
size frequencies (2014–2015 data).

Age	1	2	3
L moy	47.38	65.26	98.36
rate of growth	17.88	33.1	

Table 2. Age-length key of *Conger conger*
adjusted by the NORMSEP program.

tween age group 2 and 3 is expected to be lower than between age group 1 and 2. Thus, for the interpretation to be consistent with von Bertalanffy's model, it is assumed that there is an additional age group between age group 2 and 3, by assigning an additional year to age group 3. The plausible expla-

nation would be that the peaks corresponding to age groups 2 and 3 are represented by a large number of cohort members, while the cohort represented by the length classes between these two peaks comes from a small age group (Sparre & Venema, 1996). Similarly, length classes greater than the peak assigned to age group 3 are all grouped together in the latter. This could be explained by the small number of large individuals in our sample.

The Tomlinson & Abramson (1961) method provides a value of L∞ in the order of 147.43 cm which corresponds to a value of K in the order of 0.34 /year. This asymptotic length is less than the maximum observed value (Lmax = 174 cm), which reflects an underestimation of L∞. This can be explained by the small number of people assigned to the large individuals in the sample.

The Powell-Wetherall method (Sparre & Venema, 1996) (Fig. 4) provides a L∞ of the order of 226.96 cm. This L∞ value is incorporated into the K-Scan Label of the ELEFAN I program (Fig. 5) to estimate a corresponding K value based on the highest Rn score (ESP/ASP) (Gayanilo et al., 2005). The program provides a value of K= 0.5/year. However, this growth coefficient seems to be overestimated for the growth of a benthic species.

In the "Response Surface" subprogram of ELEFAN I (Fig. 6), the value pair L∞ = 224.4 cm and K = 0.2 year-1 seems the most appropriate, since the estimated L∞ value is higher than the Taylor approximation (Lmax/0.95 = 183.16 cm) and very close to the asymptotic length estimated by the Powell-Wetherall method which is suggested by Pauly & Moreau (1997) for a good estimate of L∞. Thus, the value of K = 0.2/year seems to be the most suitable for the growth of a benthic species, which allows us to retain these parameters for the rest of the study.

Table 3 summarizes the growth parameters obtained by the different methods used for *C. conger* (the parameters selected L∞ and K are shown in bold).

The linear growth of *C. conger* obtained by the ELEFAN I method is expressed by the relationship:

$$L_t = 224.4 * (1 - e^{-0.2*t})$$

Nevertheless, there is little information on the growth of the European conger eel, not only in the Mediterranean Sea, but also in the different regions
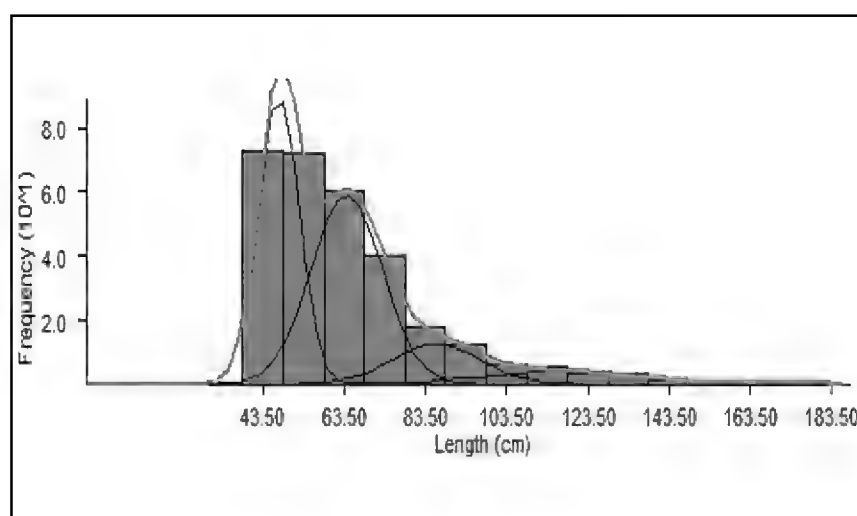
Figure 3. Adjustment of sub-populations obtained by the Bhattacharya method by the NORMSEP program.
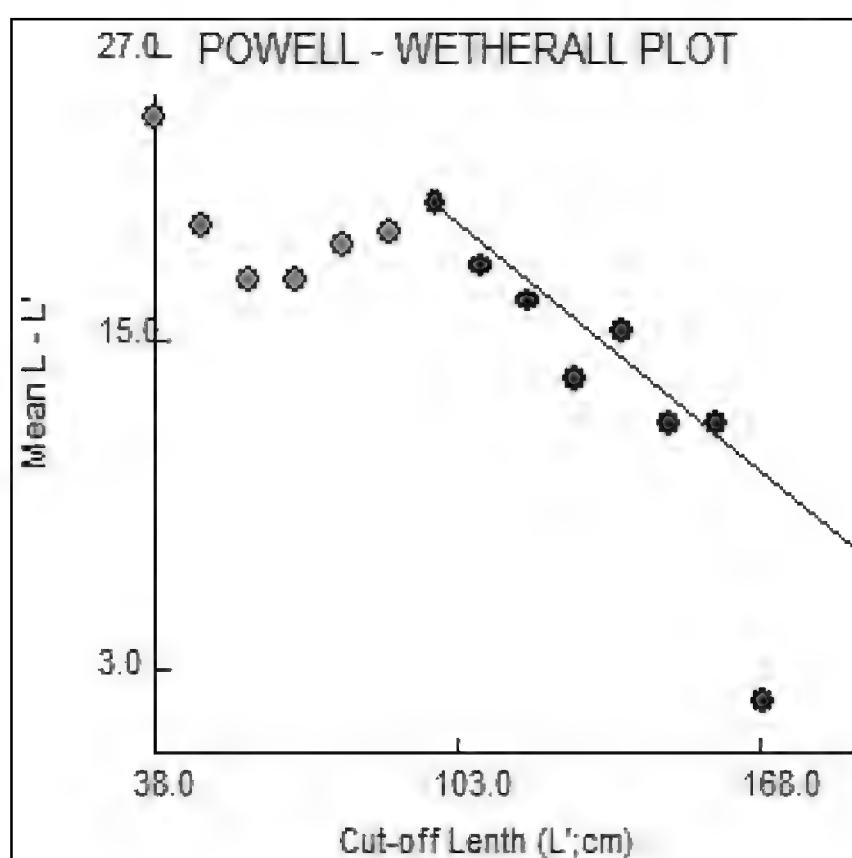

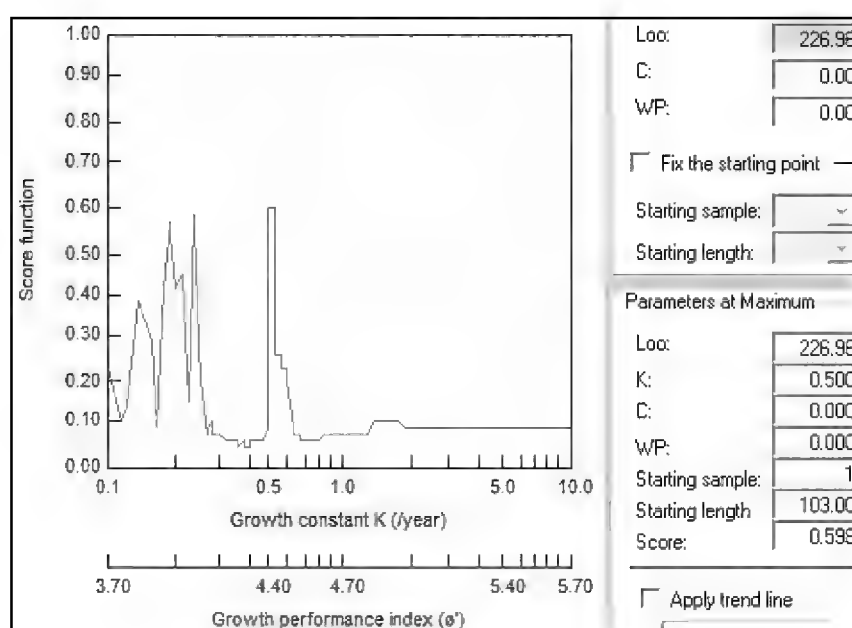Figure 4. Determination of L∞ and Z/K by the Powell-Wetherall method.

Figure 5. Variation of Rn as a function of K (K-Scan label).

of the globe, which makes the comparison of our results very limited.

We find that the asymptotic length estimated in this study is close to the results obtained by O'Sullivan et al. (2003), and Flores-Hernandez (1990) (Table 4), while the value of K is overestimated compared to the values obtained in the above studies. This may be due to the different methods used and the regions from which samples were taken. The difference between the different study regions is probably related to environmental conditions such as: food availability and water temperature (Overholtz, 1989; Jorgensen, 1992; Brander, 1995; Lorenzen & Enberg, 2002; Daufresne et al., 2009; Hunter et al., 2016).

Height-weight relationship

The height-weight relationship was performed from 183 individuals and allows us to calculate the asymptotic weight (W∞) of our species.

The results are grouped in Table 5 and figure 7.

The reduced deviation test (Schwartz, 1992) shows a significant difference between the observed allometry coefficient (b = 3.4) and the theoretical value (b0 = 3). This exposes a major allometry in *C. conger*, indicating that the weight grows faster than the length cube. However, the lack of bibliographic data on the height-weight relationship of *C. conger* makes it difficult to compare our results with the literature.

Exploitation indices

With a high regression coefficient ($r^2 = 0.98$), and eliminating the last two classes of regression, for the following reasons: a) low sample sizes; b) as we approach L∞ the relationship between age t(L) and length (L) becomes uncertain (Sparre & Venema, 1996), the total mortality Z of *C. conger* is about 1.28/year (Fig. 8).

The natural mortality rate M, estimated by the Djabali et al. (1994), is about 0.25 year^{-1}.
The fishing mortality rate on the stock of *C. conger* in the Algerian basin is deduced by the formula. The values of the different mortality rates are shown in Table 6.

The exploitation indices calculated for *C. conger* in the Algerian basin show that the current exploitation rate is around 80%, which is well above

Lmax obs (cm)	Lmax/0.95 (cm)	Tomlinson and Abramson			Powell Wetheral		ELEFAN I			
							K-Scan		Response Surface	
		L∞	K	t0	L∞	Z/K	L∞	K	L∞	K
174	183.16	147.43	0.34	- 0.31	226.96	4.64	226.96	0.5	**224.4**	**0.2**

Table 3. Summary of estimated growth parameters for *Conger conger*.

Author	Country	Region	Methodology	L∞	K	T0	Gender	W∞
O'Sullivan et al., 2003	Ireland	Irish coastal waters	Reading Otoliths	271	0.037	- 1.396	/	
O'Sullivan et al., 2003	Ireland	Irish coastal waters	Reading Vertebrae	214	0.059	- 1.96	/	
Correia et al., 2009	Portugal	Atlantic Iberian waters	Reading Otoliths	265	0.07	- 1.20	F	
Flores-Hernandez, 1990	France	Mor Braz, South Brittany	Reading Otoliths	94.8	0.27	- 0.79	M	1.6
Flores-Hernandez, 1990	France	Mor Braz, South Brittany	Reading Otoliths	229	0.068	- 2.4	F	30.4
Fannon et al., 1990	Ireland	Small Saltee Island, Wexford County	Reading Otoliths	265	0.063	- 0.39	F	-

Table 4. Growth parameters of *Conger conger* according to the literature.

Species	a	b	R²	t	W∞ (kg)
Conger conger	0.0003	3.399	0.96	7.82	27.84

Table 5. Parameters of the height-weight relationship.

Species	M (year^{-1})	Z (year^{-1})	F (year^{-1})	E (%)
C. conger	0.25	1.28	1.03	80

Table 6. Exploitation indices of *Conger conger*.

equilibrium (50%), which implies a condition of biological overexploitation of the European conger stock in the Algerian basin. On the basis of these results, and in the context of sustainable fisheries management, we suggest reducing the pressure on the European conger eel stock through both trawl fishing and recreational fishing, while reducing fishing effort, directing fishermen to other fishing grounds and/or increasing the mesh size of trawls used to fish the stock in question.

Nevertheless, a study of the state of exploitation using appropriate models is essential in order to confirm these results, to verify the proposed development scenarios and to evaluate their impacts on the level of exploitation of the European conger eel.
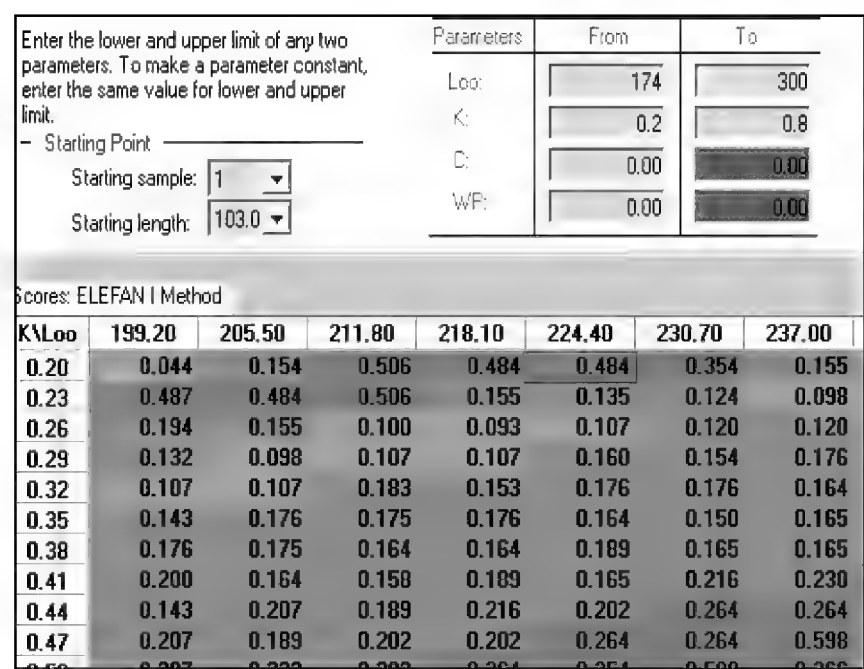
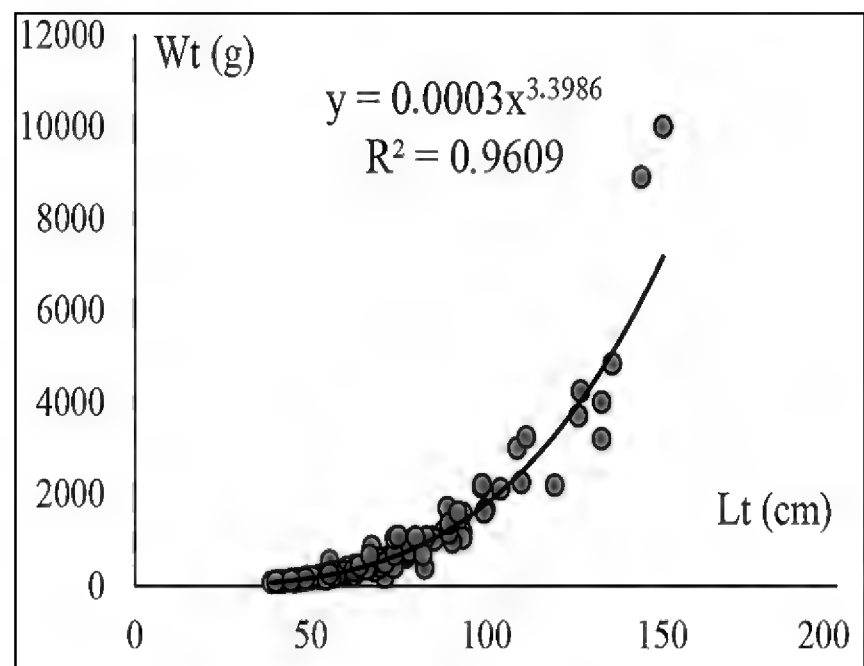Figure 6. K/L∞ torques and their respective scores (Rn) (ELEFAN I) of *Conger conger*.

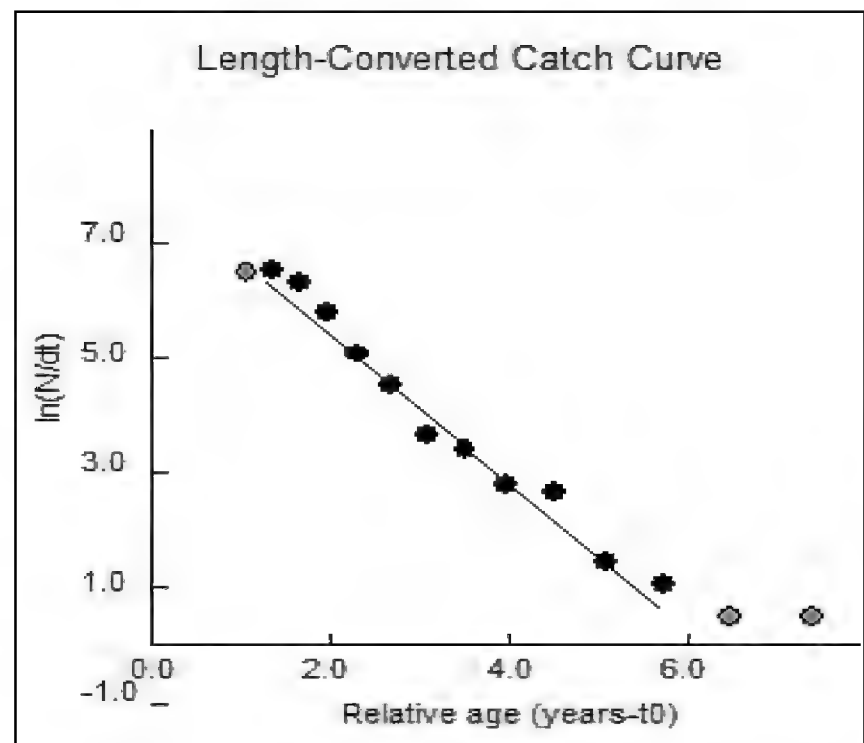Figure 7. Size and weight ratio of *Conger conger* .

Figure 8. Linearized catch curve.

CONCLUSIONS

The study of the growth of *C. conger* in the Algerian basin was carried out by analysing age structures and size structures. The growth parameters used are those obtained by the ELEFAN I method, carried out using FISAT II software, by presenting the following linear von Bertalanffy growth formula:

$$L_t = 224.4 * (1 - e^{-0.2*t})$$

Weight growth shows a major allometry in *C. conger* (b = 3.4), whose weight grows faster than the cube of the length.

The exploitation rate E = 80% shows that the conger eel stock appears to be in a state of organic overexploitation. To remedy this situation, we propose management scenarios for this fishery, based on changes in fishing effort and variations in trawl mesh size, emphasizing the need to estimate the level of exploitation of the species in question by appropriate models using the parameters estimated in this study.

This shows that the study of growth is a key step in the management of fish stocks and fisheries management, presenting the first step for estimating the level of exploitation of a given species and assessing the state of its stock.

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The biodiversity of the marine Heterobranchia fauna along the central-eastern coast of Sicily, Ionian Sea

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ABSTRACT   The first updated list of the marine Heterobranchia for the central-eastern coast of Sicily (Italy) is here reported. This study was carried out, through a total of 271 scuba dives, from 2017 to the beginning of 2020 in four sites located along the Ionian coasts of Sicily: Catania, Aci Trezza, Santa Maria La Scala and Santa Tecla. Through a photographic data collection, 95 taxa, representing 17.27% of all Mediterranean marine Heterobranchia, were reported. The order with the highest number of found species was that of Nudibranchia. Among the study areas, Catania, Santa Maria La Scala and Santa Tecla had not a remarkable difference in the number of species, while Aci Trezza had the lowest number of species. Moreover, among the 95 taxa, four species considered rare and six non-indigenous species have been recorded. Since the presence of a high diversity of sea slugs in a relatively small area, the central-eastern coast of Sicily could be considered a zone of high biodiversity for the marine Heterobranchia fauna.

KEY WORDS   diversity; marine Heterobranchia; Mediterranean Sea; sea slugs; species list.

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INTRODUCTION

Malacological studies and researches on marine Heterobranchia in Sicily (Italy) have always been scarce and fragmentary. Indeed, the last study on Sicilian marine Heterobranchia dates back to 1987 (Cattaneo Vietti & Chemello, 1987). Cattaneo and Barletta (1984) argued that taxa collected and reported in Sicily represent about 30% of all Mediterranean species. This last date may not reflect a low level of biodiversity in marine Heterobranchia, but a lack of data on this group (Cattaneo Vietti & Chemello, 1987). Moreover, studies on sea slugs in Sicily show a difference. Indeed, the eastern sector was less explored than the western sector, where

more researches were carried out (Cattaneo Vietti & Chemello, 1987).

In this work the marine Heterobranchia fauna of the central-eastern sector of Sicily (Ionian Sea) has been studied. This area, represented by Etna's eastern slopes, is geologically constituted by a complex of subvolcanic rocks, mainly consisting of columnar basalts deposited within the "pre-Etnean Gulf", and effusive marine products forming fields of pillow lavas (Cristofolini, 1975; Corsaro & Cristofolini, 1997; Sciuto et al., 2017). Accumulated along the coast and at the base of shallow cliffs, there is a belt of blocks up to a few meters in size, caused by rocky outcrops dissected by fault systems and partially dismantled (Sciuto et al.,

2017). From the coastline to down to 10-15 m depth, the bottom has a sloping topography and consists of the basaltic bedrock covered with large volcanic blocks (Sciuto et al., 2017). Offshore, the bottom of sediments becomes progressively muddier at major depths with rocky outcrops locally exposed (Rosso, 2001). The study area hosts several benthic habitats, some of which are protected by the EU Habitat Directive (92/43/EEC): pre-coralligenous and coralligenous assemblages, soft-bottom substrates, *Posidonia oceanica* (Linnaeus) Delile, 1813 meadows, and algal biocoenosis on rocky substrates.

To carry out this study six stations along the central-eastern coast of Sicily were selected, two of which are in Protected Areas. Indeed, in the site of Aci Trezza (37.561389 N; 15.157492 E) there are a Marine Protected Area (MPA) named "Ciclopi Islands", an Integral Nature Reserve called "Lachea Island and Cyclops stacks" and two Sites of Community Importance (SCIs) ("Bottoms of Acicastello" and "Cyclops Islands"), according to the Natura 2000 network. Furthermore, in the site of Santa Maria La Scala (37.617222 N; 15.172222 E) there are an Oriented Natural Reserve called "La Timpa" and a Site of Community Importance named "Timpa of Acireale" (Catra et al., 2006). Therefore, especially in these areas, knowing and preserving the biodiversity of sea slugs is extremely important and necessary. Indeed, the changes in marine Heterobranchia fauna composition may reveal environmental modifications (Furfaro et al., 2020),

caused by natural or anthropogenic factors. Moreover, a knowledge on the diversity of the marine Heterobranchia fauna can disclose the presence of any non-indigenous species. Consequently, studies focused on the production of species lists allow the hidden diversity to be revealed (Furfaro et al., 2020) and allow comparisons with different geographical areas to be made.

To date, there is not an updated list on the sea slugs of the central-eastern sector of Sicily. Indeed, data on the marine Heterobranchia fauna of the central-eastern sector of Sicily, referred to as Gulf of Catania and provinces, are ancient and reported only 5 species (Philippi, 1836-1844; Aradas, 1847; Cattaneo Vietti & Chemello, 1987). For this reason, the aim of this work was to contribute to the knowledge of the marine Heterobranchia present in this area, which has always been poorly explored, and to document the presence of rare and non-indigenous species.

MATERIAL AND METHODS

This study was carried out from January 2017 to March 2020, in 6 stations located along the central-eastern sector of Sicily (Figs. 1, 2). These sites were selected based on different environmental conditions. Two stations, "Ognina" (37.530528 N; 15.120028 E) and "Bellatrix" (37.533917 N; 15.126778 E), are situated close to each other in the municipality of Catania. These sites are sub-
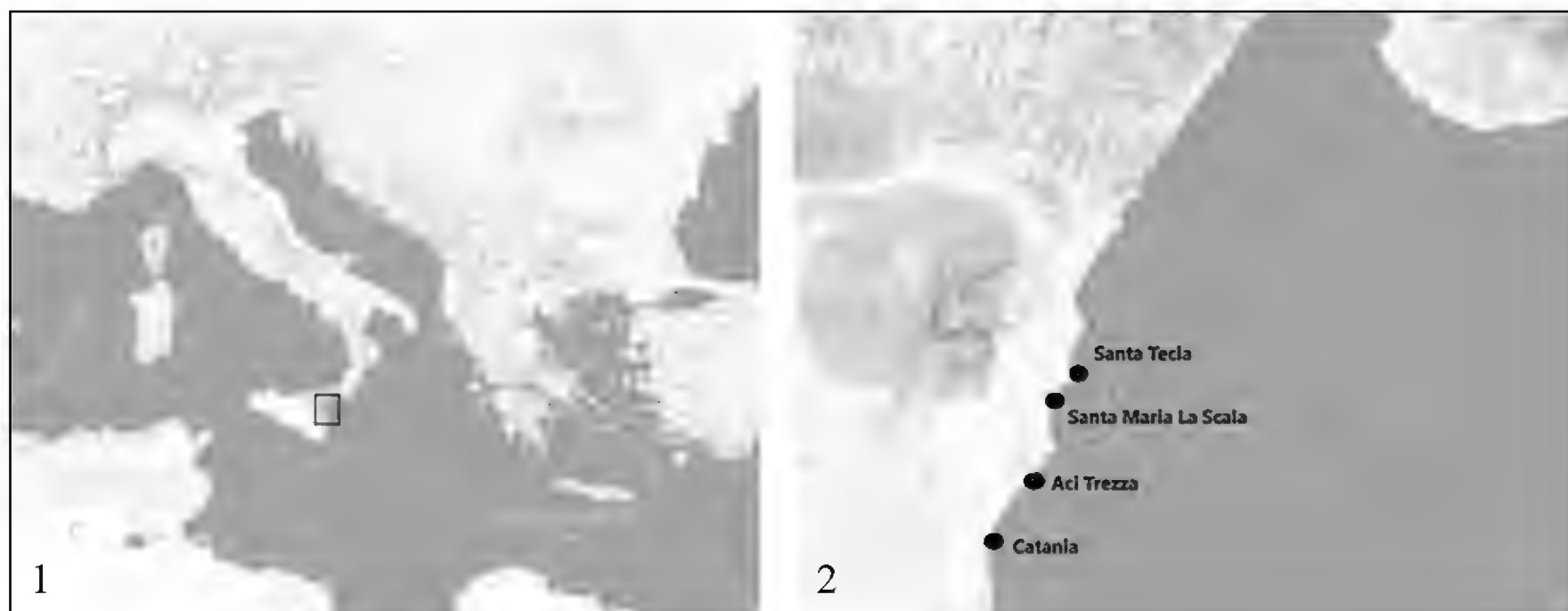
Figures 1, 2. Geographical area in the Ionian Sea (Mediterranean Sea) where this study was carried out: central-eastern sector of Sicily, where are located the study areas (Fig. 1); detail of the study areas (Fig. 2).

jected to a strong anthropization, due to the proximity of a harbour and the exploitation of the coast. Since these sites have similar ecological conditions, they were considered as a single site, listed from now on as "Catania". Another station called "Padre Pio" (37.566083 N; 15.166361 E) is located in the zone "B", the general reserve zone, of the MPA of "Ciclopi Islands", in the hamlet of Aci Trezza, within the municipality of Acicastello. This site, especially during summer, has a strong anthropic pressure. From now on, for simplification this area will be referred as "Aci Trezza". A station named "Testa dell'acqua" (37.6125 N; 15.175389 E) is located in the hamlet of Santa Maria La Scala and, thus, it will be simply listed as "Santa Maria La Scala". Finally, two sites, "Scalo Pennisi" (37.639778 N; 15.184722 E) and "Acque Fredde" (37.637583 N; 15.181167 E), are situated in the hamlet of Santa Tecla. These three stations are all located in the municipality of Acireale. Moreover, in these three sites there are several springs due to the flow of freshwater from the Etna to the sea (Ferrara, 1977; Catra et al., 2006). Since the stations of "Scalo Pennisi" and "Acque Fredde" are situated close to each other and are the least affected by anthropogenic factors, they considered as a single site, listed from now on as "Santa Tecla". Instead, the site of "Santa Maria La Scala" was considered individually because it has intermediate conditions between those of Catania and Santa Tecla.

Throughout the years of study, a total of 271 scuba dives was carried out: 92 in "Catania", 81 in "Santa Maria La Scala", 74 in "Santa Tecla", 24 in "Aci Trezza". The scuba dives (0 - 45 m) were conducted all year round (marine-weather conditions permitting), twice a week, during daylight, between 9-11:30 am. Occasionally, some dives were carried out at night in the site of Aci Trezza. In each site, the same pathway has always been followed. Data on marine Heterobranchia fauna were collected with the "photographic capture" technique: during each scuba dive all sea slugs were photographed with an Olympus TG-4 Underwater Camera, were counted and the depth, where they were encountered, was annotated. Subsequently, the photographs with the specimens were used for the identification of the species, by consulting Schmekel & Portmann (1982), Thomson & Brown (1984), Betti (2011), Trainito & Doneddu

(2014) and Sea Slug Forum (Rudman, 1999). The validity of the species names has been checked by consulting the World Register of Marine Species (Horton et al., 2020). Finally, the collected data were processed in Excel to create a table and graphs.

RESULTS

Through data collection, a total of 95 taxa of marine Heterobranchia (Table 1) were found, consisting of: 1 Rhodopoidea, 4 Pleurobranchida, 61 Nudibranchia, 2 Umbraculida, 7 Aplysiida, 5 Cephalaspidaea, 1 Runcinida, 14 Sacoglossa. Their percentages are shown in figure 3. Among these 95 taxa, 75 were found in Catania, 43 in Aci Trezza, 74 in Santa Maria La Scala, 71 in Santa Tecla (Fig. 4).

Since the highest percentage of species belongs to the order Nudibranchia (64%), the differences within this order have been highlighted and it has been seen that 56% of them are represented by the suborder Cladobranchia and 44% by the suborder Doridina. Within the suborder Cladobranchia (Fig. 5), a total of 34 species was found, consisting of 12 families. Instead, within the suborder Doridina (Fig. 6) a total of 27 species was found, consisting of 9 families.

In Table 1 all sea slug species, which were found throughout this study, are reported. The asterisks present in the table represent the finding of only the eggs of a species in an area.
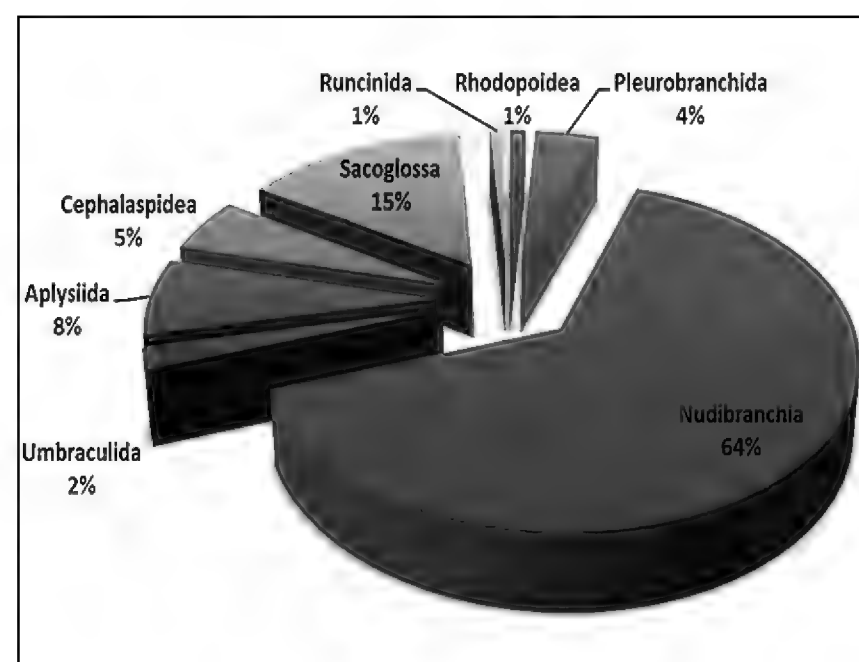

Figure 3. Percentages of the marine Heterobranchia groups found throughout the study.

Taxa	Catania	Acitrezza	Santa Maria La Scala	Santa Tecla	Depth
"Lower Heterobranchia"					
Superfamily Rhodopoidea Ihering, 1876					
Family Rhodopidae Ihering, 1876					
Rhodope sp.	X		X	X	**6.8-28 m**
Euthyneura					
Order Pleurobranchida					
Family Pleurobranchidae Gray, 1827					
Berthella cf. *aurantiaca* (Risso, 1818)	X				**6 m**
Berthella ocellata (Delle Chiaje, 1830)	X	X		X	**15-34.2 m**
Pleurobranchus testudinarius Cantraine, 1835	X	X	*	*	**5-45 m**
Pleurobranchus wirtzi Ortea, Moro & Caballer, 2014	X				**6.6 m**
Order Nudibranchia					
Suborder Doridina					
Family Calycidorididae Roginskaya, 1972					
Diaphorodoris alba Portmann & Sandmeier, 1960	X	X	X	X	**12-37 m**
Diaphorodoris papillata Portmann & Sandmeier, 1960	X	X	X	X	**7-37 m**
Family Goniodorididae H. Adams & A. Adams, 1854					
Okenia problematica Pola, Paz-Sedano, Macali et al., 2019	X				**14.5-17.4 m**
Trapania lineata Haefelfinger, 1960	X		X	X	**5.3-35 m**
Family Polyceridae Alder & Hancock, 1845					
Kaloplocamus ramosus (Cantraine, 1835)		X			**15 m**
Polycera quadrilineata (O.F. Müller, 1776)	X	X	X	X	**6-37 m**
Family Aegiridae P. Fischer, 1883					
Aegires leuckartii Vérany, 1853	X			X	**6-17.1 m**
Aegires sublaevis Odhner, 1932	X				**5,7 m**
Family Chromodorididae Bergh, 1891					
Felimare fontandraui (Pruvot-Fol, 1951)	X	X	X	X	**9-37 m**
Felimare gasconi (Ortea, 1996)	X		X		**6.1-19.2 m**
Felimare orsinii (Vérany, 1846)			X		**25,6 m**
Felimare picta (Philippi, 1836)	X	X	X	X	**4-36.6 m**
Felimare tricolor (Cantraine, 1835)	X	X	X	X	**7-37.2 m**
Felimare villafranca (Risso, 1818)	X		X	X	**8-30 m**
Felimida binza (Ev. Marcus & Er. Marcus, 1963)	X	X	X	X	**6-34 m**
Felimida krohni (Vérany, 1846)	X	X	X	X	**6-36 m**
Felimida luteorosea (Rapp, 1827)	X		X	X	**10-35 m**
Felimida purpurea (Risso, 1831)	X		X	X	**8-35.5 m**

Taxa	Catania	Acitrezza	Santa Maria La Scala	Santa Tecla	Depth
Family Dorididae Rafinesque, 1815					
Doris verrucosa Linnaeus, 1758		X			**< 1 m**
Family Discodorididae Bergh, 1891					
Baptodoris cf. *cinnabarina* Bergh, 1884	X				**5,7 m**
Jorunna tomentosa (Cuvier, 1804)		X			**< 1 m**
Peltodoris atromaculata Bergh, 1880	X	X	X	X	**10-37 m**
Platydoris argo (Linnaeus, 1767)	X		X	X	**< 1-34 m**
Taringa tritorquis Ortea, Perez & Llera, 1982	X		X		**6.9-9.5 m**
Family Phyllidiidae Rafinesque, 1814					
Phyllidia flava Aradas, 1847	X	X	X		**12-37 m**
Family Dendrodorididae O'Donoghue, 1924 (1864)					
Dendrodoris limbata (Cuvier, 1804)	X	X		X	**3.7-28.4 m**
Doriopsilla areolata Bergh, 1880			X	X	**5-8.7 m**
Suborder Cladobranchia					
Family Tritoniidae Lamarck, 1809					
Tritonia manicata Deshayes, 1853			X		**34 m**
Tritonia striata Haefelfinger, 1963		X			**17 m**
Family Dotidae Gray, 1853					
Doto acuta Schmekel & Kress, 1977	X		X	X	**6-34 m**
Doto cervicenigra Ortea & Bouchet, 1989	X			X	**15-20 m**
Doto floridicola Simroth, 1888			X	X	**17.5-32.5 m**
Family Janolidae Pruvot-Fol, 1933					
Antiopella cristata (Delle Chiaje, 1841)	X	X	X	X	**11-36 m**
Family Flabellinidae Bergh, 1889					
Calmella cavolini (Vérany, 1846)	X	X	X	X	**4-37 m**
Edmundsella pedata (Montagu, 1816)	X	X	X	X	**3-37 m**
Flabellina affinis (Gmelin, 1791)	X	X	X	X	**8-38 m**
Paraflabellina ischitana (Hirano & T. E. Thompson, 1990)	X		X	X	**5-37 m**
Family Coryphellidae Bergh, 1889					
Fjordia lineata (Lovén, 1846)	X		X	X	**8,5-34 m**
Family Samlidae Korshunova, Martynov et al., 2017					
Luisella babai (Schmekel, 1972)	X	X	X	X	**20-37 m**
Family Eubranchidae Odhner, 1934					
Amphorina farrani (Alder & Hancock, 1844)	X		X	X	**5-16 m**
Capellinia doriae Trinchese, 1874			X		**9.1 m**
Eubranchus tricolor Forbes, 1838				X	**9 m**
Family Trinchesiidae F. Nordsieck, 1972					
Trinchesia caerulea (Montagu, 1804)	X		X	X	**6.5-37 m**

Taxa	Catania	Acitrezza	Santa Maria La Scala	Santa Tecla	Depth
Trinchesia foliata (Forbes & Goodsir, 1839)	X	X	X	X	6-27 m
Trinchesia genovae (O'Donoghue, 1926)	X	X	X	X	5-35 m
Trinchesia ocellata Schmekel, 1966	X		X	X	17-36 m
Family Embletoniidae Pruvot-Fol, 1954					
Embletonia pulchra (Alder & Hancock, 1844)	X		X	X	10-6 m
Family Facelinidae Bergh, 1889					
Caloria elegans (Alder & Hancock, 1845)	X		X		21.3-27.8 m
Cratena peregrina (Gmelin, 1791)	X	X	X	X	4-37 m
Dicata odhneri Schmekel, 1967	X	X	X	X	4.5-32 m
Facelina annulicornis (Chamisso & Eysenhardt, 1821)	X		X	X	7-10 m
Facelina rubrovittata (Costa A., 1866)	X	X	X	X	5-32 m
Facelinopsis marioni (Vayssière, 1888)	X	X	X	X	3-22 m
Favorinus branchialis (Rathke, 1806)	X	X	X	X	5-37 m
Family Myrrhinidae Bergh, 1905					
Dondice banyulensis Portmann &Sandmeier, 1960	X		X	X	8-38 m
Dondice trainitoi Furfaro & Mariottini, 2020			X		20 m
Godiva quadricolor (Barnard, 1927)			X		7 m
Family Aeolidiidae Gray, 1827					
Berghia coerulescens (Laurillard, 1832)	X		X	X	8-36 m
Berghia verrucicornis (A. Costa, 1867)	X				4,8 m
Limenandra nodosa Haefelfinger & Stamm, 1958			X	X	5-17 m
Spurilla neapolitana (Delle Chiaje, 1841)	X	X	X	X	5-20 m
Order Umbraculida					
Family Umbraculidae Dall, 1889 (1827)					
Umbraculum umbraculum (Lightfoot, 1786)		X	X	X	5.1-36 m
Family Tylodinidae Gray, 1847					
Tylodina perversa (Gmelin, 1791)	X		X	X	12-31 m
Order Aplysiida					
Family Aplysiidae Lamarck, 1809					
Aplysia dactylomela Rang, 1828	X	X	X	X	< 1-19 m
Aplysia depilans Gmelin, 1791	X		X	X	2.9-23 m
Aplysia fasciata Poiret, 1789	X		X	X	2-25.3 m
Aplysia parvula Mörch, 1863	X	X	X	X	2.9-17 m
Aplysia punctata (Cuvier, 1803)	X	X	X	X	1.4-21.7 m
Notarchus punctatus Philippi, 1836	X	X	X	X	6-21,5 m
Phyllaplysia cf. *lafonti* (P. Fischer, 1870)	X		X	X	5-18 m
Order Cephalaspidea					
Family Aglajidae Pilsbry, 1895 (1847)					

Taxa	Catania	Acitrezza	Santa Maria La Scala	Santa Tecla	Depth
Aglaja tricolorata Renier, 1807			X	X	**11.2-15 m**
Biuve fulvipunctata (Baba, 1938)				X	**4.4 m**
Camachoaglaja africana (Pruvot-Fol, 1953)	X	X		X	**< 1 - 7 m**
Family Haminoeidae Pilsbry, 1895					
Haloa sp.	*	X	*	X	**3-33 m**
Lamprohaminoea cyanomarginata (Heller & T.E. Thompson, 1983)	X	X	X	X	**5-25 m**
Order Runcinida					
Family Runcinidae H. Adams & A. Adams, 1854					
Runcina sp.	X		X		**6-7.2 m**
Superorder Sacoglossa					
Family Oxynoidae Stoliczka, 1868 (1847)					
Lobiger serradifalci (Calcara, 1840)			X	X	**5-10 m**
Oxynoe olivacea Rafinesque, 1814				X	**6-7.6 m**
Family Plakobranchidae Gray, 1840					
Bosellia mimetica Trinchese, 1891	X	X	X	X	**1 -38 m**
Elysia hetta Perrone, 1990	X		X		**21-30 m**
Elysia margaritae Fez, 1962	X	X	X		**5-30 m**
Elysia rubeni Martín-Hervás, Carmona et al., 2019	X	X	X	X	**7-21 m**
Elysia timida (Risso, 1818)	X	X	X	X	**1-18 m**
Elysia translucens Pruvot-Fol, 1957			X	X	**6.8-7.5 m**
Elysia viridis (Montagu, 1804)	X		X	X	**7-22 m**
Thuridilla hopei (Vérany, 1853)	X	X	X	X	**1 - 36 m**
Family Limapontiidae Gray, 1847					
Placida cremoniana (Trinchese, 1892)	X				**1.4 m**
Placida viridis (Trinchese, 1874)				X	**7.2 m**
Family Hermaeidae H. Adams & A. Adams, 1854					
Hermaea bifida (Montagu, 1816)	X			X	**6-19 m**
Hermaea variopicta (A. Costa, 1869)	X		X		**7 m**

Table 1. List of marine Heterobranchia found in four areas along the central-eastern sector of Sicily (Ionian Sea). Abbreviation cf. is from the Latin confer/conferatur, both meaning compare.

DISCUSSION

The present study, which was carried out from 2017 to the beginning of 2020 in four areas located along the central-eastern sector of Sicily, reports the presence of 95 taxa of marine Heterobranchia (Table 1). Through data collection, it was observed that the highest number of the found species belongs to the order Nudibranchia, with a percentage of 64% (Fig. 3). Moreover, within this order the majority of the species belongs to the suborder Cladobranchia (56%), in which the family with the highest number of species was that of Facelinidae Bergh, 1889 (Fig. 5). Instead, the suborder Doridina represented 44% of the Nudibranchia. In this suborder,
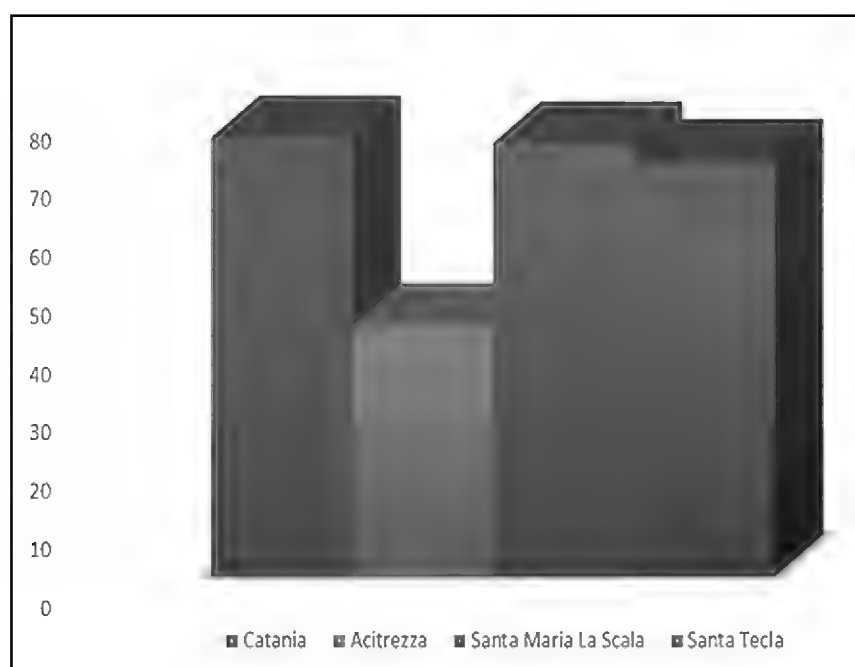
Figure 4. Number of taxa found in the
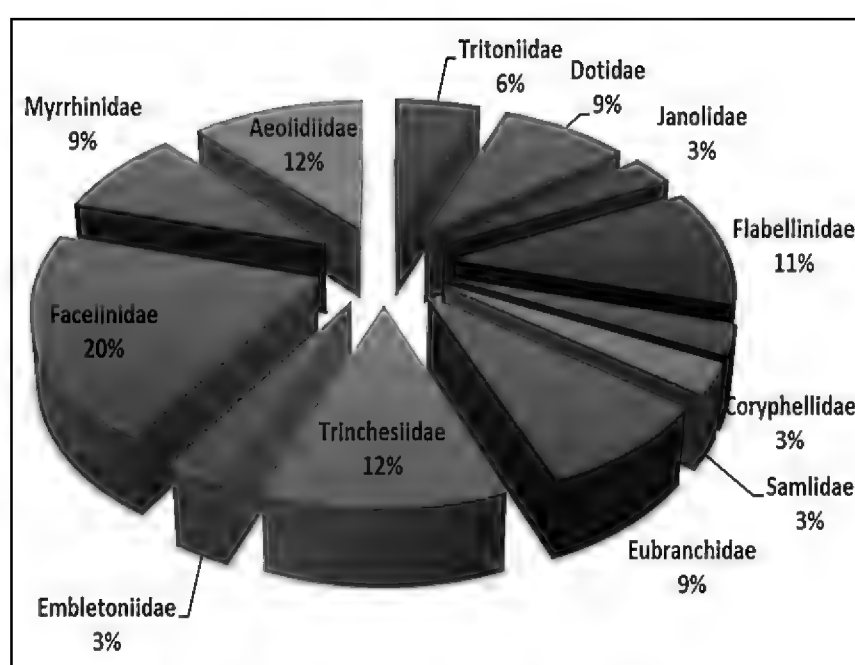different study areas.

Figure 5. Percentages of the families of the suborder
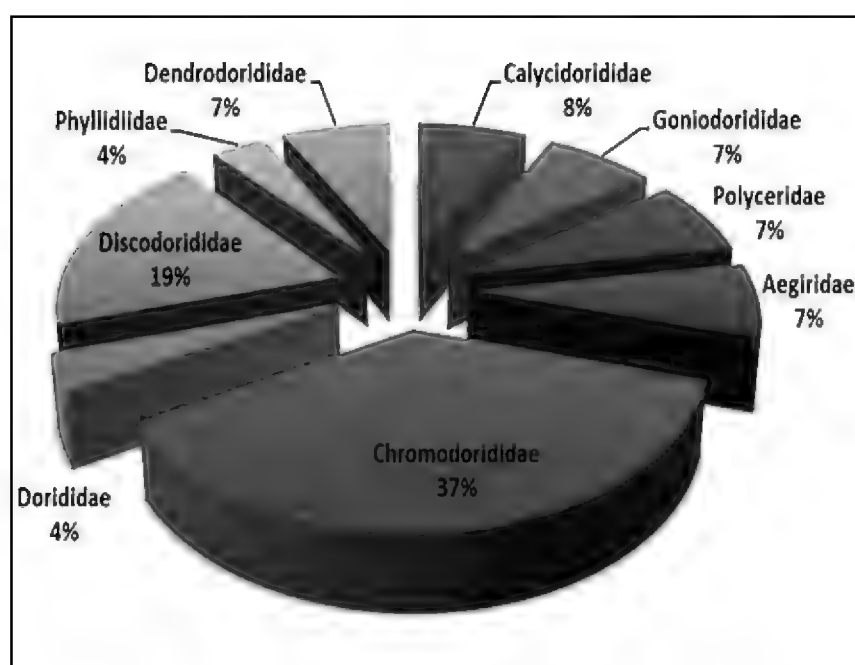Cladobranchia (Nudibranchia) seen during this study.

Figure 6. Percentages of the families of the suborder
Doridina (Nudibranchia) seen during this study.

the family with most species was that of Chro-
modorididae Bergh, 1891 (Fig. 6).

Regarding the distribution of the sea slug taxa
in the different study areas (Fig. 4), it was not ob-
served a remarkable difference in the number of the
found species in the sites of Catania, Santa Maria
La Scala and Santa Tecla. Instead, in the site of Aci
Trezza the lowest number of species has been
found. Although in this area fewer scuba dives were
conducted than the other sites, the authors have ex-
tended knowledge of this area before the present
study and believe that the lower number of species
is related to other factors. Indeed, the site of Aci
Trezza presents a homogeneous topography and is
characterized by shallower sea bottoms, while the
remaining study areas have a sloping topography
and deeper sea bottoms. Therefore, this last type of
topography allows the presence of a higher number
of microhabitats and more variable environmental
conditions.

Through data collection, for the first time it has
been realized a list of the marine Heterobranchia pre-
sent in the central-eastern coast of Sicily. Among the
95 taxa here reported, four species considered rare
and five non-indigenous species have been found.
Among the rare species, the following are reported:
Elysia rubeni Martín-Hervás, Carmona, K. R.
Jensen, Licchelli, Vitale & Cervera, 2019
(Gerovasileiou et al., 2020), *Okenia problematica*
Pola, Paz-Sedano, Macali, Minchin, Marchini,Vitale,
Licchelli et Crocetta, 2019 (Lombardo & Marletta,
2020a), *Taringa tritorquis* Ortea, Perez & Llera, 1982
(Gerovasileiou et al., 2020; Lombardo & Marletta,
2020b) and *Dondice trainitoi* Furfaro & Mariottini,
2020 (Gerovasileiou et al., 2020). Instead, regarding
non-indigenous species, the following taxa are here
reported: *Aplysia dactylomela* Rang, 1828 (Valdés et
al., 2013), *Lamprohaminoea cyanomarginata* (Heller
& T. E. Thompson, 1983) (Crocetta & Vazzana,
2009), *Godiva quadricolor* (Barnard, 1927) (Lom-
bardo & Marletta, 2019a), *Biuve fulvipunctata* (Baba,
1938) (Lombardo & Marletta, 2020b), and *Pleuro-
branchus wirtzi* Ortea, Moro & Caballer, 2014 (Lom-
bardo & Marletta, 2019b). Moreover, six species,
whose identification is doubtful, are reported in Table
1 and in figures 7–12: *Rhodope* sp., *Berthella* cf. *au-
rantiaca* (Risso, 1818), *Baptodoris* cf. *cinnabarina*
Bergh, 1884, *Phyllaplysia* cf. *lafonti* (P. Fischer,
1870), *Runcina* sp., *Haloa* sp.

In conclusion, the present study on the marine
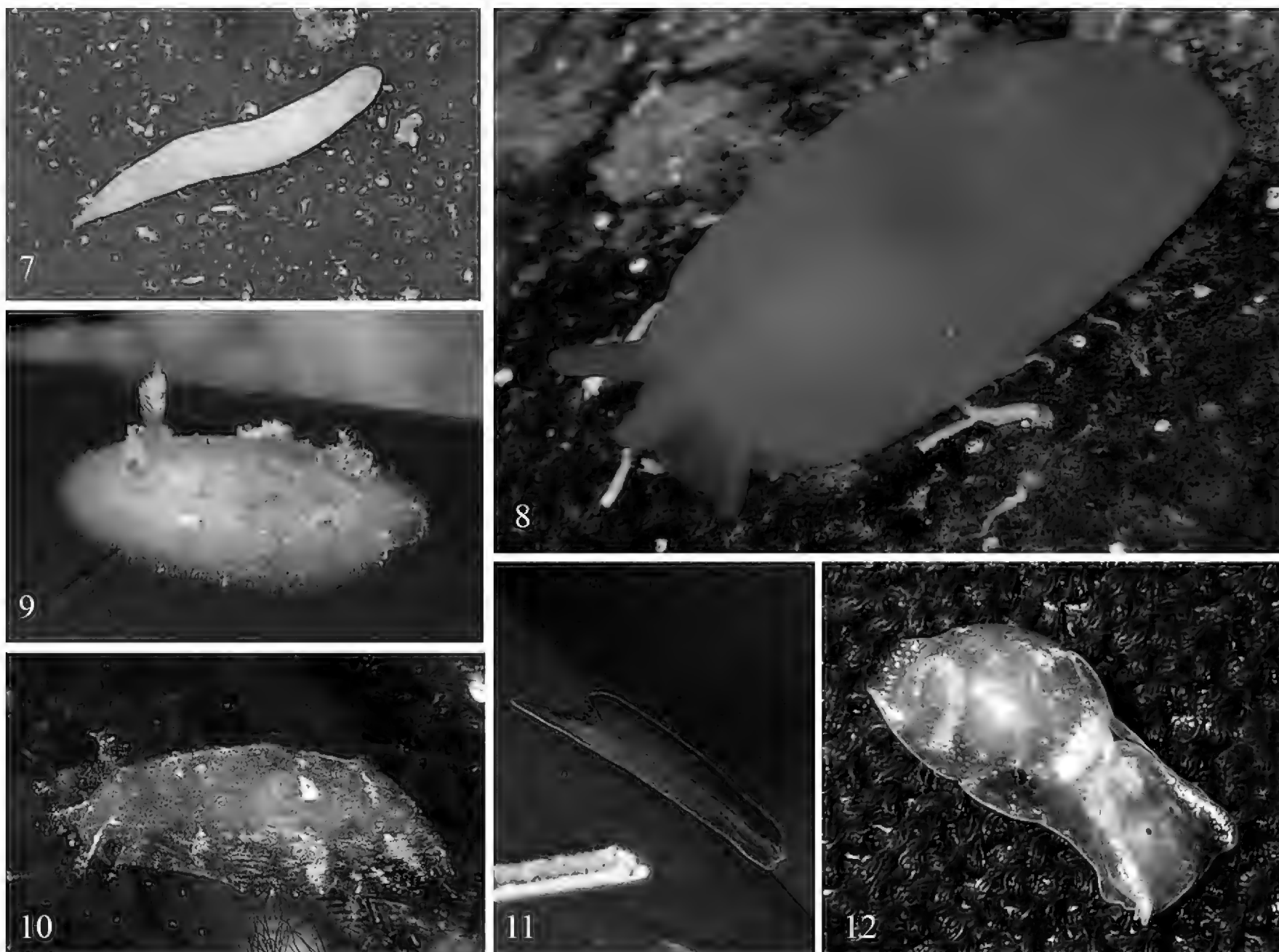
Figures 7–12. Species whose identification is doubtful: Fig. 7) *Rhodope* sp.; Fig. 8) *Berthella* cf. *aurantiaca*; Fig. 9) *Baptodoris* cf. *cinnabarina*; Fig. 10) *Phyllaplysia* cf. *lafonti*; Fig. 11) *Runcina* sp.; Fig. 12) *Haloa* sp. (photos A. Lombardo).

Heterobranchia of the central-eastern coast of Sicily, represents the first contribution to the knowledge of this group in this area, that has always been poorly studied. Moreover, this study revealed high biodiversity in the marine Heterobranchia present in this area. Indeed, the found taxa represent 17.27% of all Mediterranean sea slugs, which in total are about 550 (Trainito & Doneddu, 2014; Furfaro et al., 2020). Therefore, although the small examined area, the central-eastern coast of Sicily could be considered a zone of high biodiversity for the marine Heterobranchia fauna. Future studies will be focused on the frequency, abundance and seasonality of the species distributed in the examined areas.

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First record of the crab *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Brachyura Portunidae) off Favignana (Sicily, Italy)

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ABSTRACT   The record of the blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Brachyura Portunidae) off Favignana (Aegadian Islands, Sicily, Italy) at 60–70 m depth is reported. A possible hypothesis on the unusual depth of collection is given.

KEY WORDS   *Callinectes sapidus*; Favignana; alien species; Mediterranean Sea.

INTRODUCTION

The European *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Brachyura Portunidae), native to the North American Atlantic Ocean (Galil et al., 2002), has been introduced into the Mediterranean Sea probably through ballast waters (Holthuis & Gottlieb, 1955) and it first colonized the eastern part (Galil, 2011). Afterwards, numerous papers reported its presence in several places in the Mediterranean Sea such as Turkey, Israel, Greece, Croatia, Algeria, Spain, and France, in coastal waters and from lagoons or estuaries (all the localities are resumed in Labrune et al., 2019).

In Italy, its first finding dates back to 1949 and 1951 in the Venice lagoon (Giordani Soika, 1951; Mizzan, 1993), then it has been reported in other Adriatic localities, from brackish and coastal waters (Mizzan, 1993; Scaravelli & Mordenti, 2007; Florio et al., 2008; Giansante, 2012; Castriota et al., 2012; Mancinelli et al., 2013; Cilenti et al., 2015; Manfrin et al., 2016; Pagliara & Mancinelli, 2018). Other specimens were collected from the Gulf of Genova

(Suaria et al., 2017), Tuscany (Bisconti & Silvi, 2005), Sardinia (Piras et al., 2019; Culurgioni et al., 2020) and the Ionian Sea, in Apulia (Gennaio et al., 2006), Basilicata (Stasolla & Innocenti, 2014) and Calabria (Sperone et al., 2015) regions. Cerri et al. (2020) resumed the finding of the blue crabs in Italy based on the observations of correspondents, but for Sicily, it was reported only off Palermo.

The species is euryhaline and in North America lives in estuaries and marine embayments from the water edge to approximately 90 m depth (mainly at depths <35 m) on muddy and sandy bottoms (Hill et al., 1989). It is worth mentioning that in the Mediterranean localities, *C. sapidus* are found in numbers in similar habitats, mainly in estuaries and lagoons, but not in high sea.

MATERIAL AND METHODS

The study area is placed in Favignana (Aegadian Islands, Sicily, Italy).

The sample was captured in gill and trammel

nets, photographed, then frozen and preserved in 70% ethanol, and deposited in the Casamuseo "Matteo Sercia" in Favignana island (collection number CCGS 0037).

RESULTS

An adult male specimen of *C. sapidus* (carapace width 94.0 mm, carapace length 50.2 mm, wet weight 75 g) was captured in gill and trammel nets (mesh 70 mm), at south Favignana Islands, 700–800 meters depth west Secca del Toro (Sicily, Italy), on 3 August 2020, at 60–70 meters depth, on a sandy-rocky bottom (Fig. 1).

For its identification, it has been compared to a male specimen (carapace width 156.0 mm, carapace length 80.3 mm; CCGS 0038) from Sottomarina beach, near Chioggia, Venice Lagoon, obtained and offered by M. El Assil in September 2020.

Figure 1. *Callinectes sapidus*, male, collected at south Favignana (Aegadian Islands, Sicily), 700–800 m west Secca del Toro, Sicily, Italy (CCGS 0037) (photo G. Sercia).

DISCUSSION AND CONCLUSIONS

The present record from Favignana island is not unexpected given the species' presence in southern Sicily (Stagnone di Marsala, Trapani: Comune di Marsala, 2015; Agrigento: Insacco & Zava, 2017; Lampedusa Island: Cerri et al., 2020; Gulf of Castellammare, Palermo: Pipitone et al., 2020; Strait of Sicily: Falsone et al., 2020), however, the depth at which it has been caught is surprising, as it was fished in open sea.

The plausible hypothesis of this finding can be ascribed to the fact that anglers are fishing with crab traps *Carcinus aestuarii* Nardo, 1847 (Decapoda Portunidae) in the Stagnone di Marsala, where *C. sapidus* is commonly sharing the habitat with this autochthonous portunid crab (Comune di Marsala, 2015), and use them as baits for octopuses off Favignana (between 30 and 50 m depth) (Giacomo Campo, personal communication). Probably one specimen was among the *C. aestuarii* baits and escaped from the crab net, being, as a portunid, an excellent swimmer. In literature, only Daban et al. (2016) observed a single *C. sapidus* at 220 m depth from the North Aegean Sea, but any possible explanation of this finding was hypothesized.

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First record of *Aporodoris millegrana* (Alder et Hancock, 1854) (Gastropoda Heterobranchia Nudibranchia) in the Ionian Sea, central Mediterranean Sea

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ABSTRACT   The first record of *Aporodoris millegrana* (Alder et Hancock, 1854) (Gastropoda Heterobranchia Nudibranchia) in the Ionian Sea (central Mediterranean Sea) is here presented. This species was originally described for the British Isles and then, under different synonyms, was reported for the Atlantic coasts of France, Canary Islands, Madeira and the Strait of Gibraltar. In the Mediterranean Sea, this species has been only reported for different localities along the Catalan coast. This record is based on the finding of a *A. millegrana* specimen in a station along the central-eastern coast of Sicily (Italy). The individual was found under a stone at a depth of 6.4 m on a rocky seabed. In the last years, some Atlantic Nudiplera, which were never reported before, have been increasingly found in the Ionian Sea. In view of the rising water temperature in the Mediterranean Sea, the Atlantic termophilic species could settle in new areas of the Mediterranean. Nevertheless, it is not excluded that maybe *A. millegrana*, since is rare and with a sciaphilous behaviour, could be underestimated until now in the Mediterranean Sea.

KEY WORDS   *Aporodoris millegrana*; Mediterranean Sea; Sicily; *Taringa fanabensis*; *Taringa tarifaensis*.

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INTRODUCTION

Aporodoris millegrana (Alder et Hancock, 1854) is a nudibranch belonging to the family Discodorididae Bergh, 1891. Overall, this species had a troubled taxonomic history. Indeed, previously, this species was included in different genera: *Doris* Linnaeus, 1758, *Archidoris* Bergh, 1878, *Discodoris* Bergh, 1877, *Taringa* Er. Marcus, 1955 and *Thordisa* Bergh, 1877 (Dayrat, 2010; Ballesteros et al., 2016). Moreover, two species, *Taringa fanabensis* Ortea et Martínez, 1992 and *T. tarifaensis* García-Gómez et al., 1993, described for Canary Islands and Cadiz, respectively, are nowadays con-

sidered as synonyms of *A. millegrana* (Valdés & Gosliner, 2001). Nevertheless, according to Dayrat (2010) these synonyms could be possible but further studies would be needed to confirm this hypothesis. Indeed, more information are necessary to understand the infra-specific and inter-specific variations of morphological features of the species of the genera *Aporodoris* Ihering, 1886 and *Taringa*, in the Atlantic Ocean and the Mediterranean Sea (Dayrat, 2010).

Aporodoris millegrana was originally described by Alder & Hancock (1854) for Torbay (British Isles) as "*Doris millegrana*". Then, this species was found along the French coasts near Wimereux

(Bouchet & Tardy, 1976). Subsequently, it was reported for Tenerife (Canary Islands) by Ortea & Martinez (1992), as "*Taringa fanabensis*", and for Tarifa (Cadiz, Strait of Gibraltar), by García-Gomez et al. (1993), as "*Taringa tarifaensis*". In 1999, this species was found in Madeira and was reported as "*Taringa* cf. *fanabensis*" (Wirtz, 1999). In 2001, it was found in Canary Islands by Ortea et al. (2001) (as "*Taringa fanabensis*") and in Madeira by Malaquias et al. (2001) (as "*Taringa* cf. *fanabensis*"). Then, Moro et al. (2003) reported this species another time for Canary Island as "*Taringa fanabensis*". Finally, Ballesteros et al. (2016) observed this species in different localities (Cadaqués, Begur and l'Ametlla de Mar) of the Catalan coast. Consequently, *A. millegrana* in the Mediterranean Sea, was reported only along the Spanish coasts. The present report, thus, represents the first record of *A. millegrana* in Ionian Sea (central Mediterranean Sea).

MATERIAL AND METHODS

The present study was carried out in a station called Santa Maria La Scala (37,6125 N; 15,175389 E), located along the central-eastern coast of Sicily (Italy). This fishing town is sited in the municipality of Acireale and hosts a small harbour. Santa Maria La Scala is situated along Etna eastern slopes (Sciuto et al., 2017) and presents a coastal scarp extended for 6 km, denominated "Timpa". In this station there are an Oriented Natural Reserve called "La Timpa" and a Site of Community Importance (SCI), named "Timpa of Acireale" (Catra et al., 2006). Due to the high permeability of Etna's vulcanites and to the flow of freshwaters from Etna to the sea, there are several springs (Catra et al., 2006). Moreover, the seabed, where the specimen was found, has a sloping topography and is characterized by basaltic outcrops. *Aporodoris millegrana* specimen was identified *in vivo* and photographed through an Olympus TG-4 Underwater Camera, during a scuba diving, between 9–11:30 a.m., conducted by the authors.

RESULTS

On 19th May 2020, an *A. millegrana* specimen

(Figs. 1–4) was found under a stone with sponges and bryozoans, at a depth of 6.4 m. The specimen was about 20 mm long, with a flattened appearance. The individual had a violet-brownish body, with a dense pattern of tubercles on the notum. Along the edge of notum there were small bright white spots. Moreover, the notum was characterized by scattered white lines and darker patches. On each side of the visceral mass there was a small group of more evident white-yellowish tubercles. Rhinophores, yellowish with brown stripes, presented lamellae and were mucronate at the tip. The gills had the same colouring of the rhinophores. The edge of rhinophore and gill sheaths had white spiculous tubercles.

DISCUSSION

The specimen of *A. millegrana* (Figs. 1–4) reported in this study was similar to that described by Moro & Ortea (2012). Indeed, they observed that the body colour of this species is brown-violet with distinctive tubercles regularly arranged on the notum. In lateral view the body has a flattened shape. Rhinophores have greyish peduncles with 14 brownish lamellae and are mucronate at the tip. The rhinophore sheaths have tubercles, which are similar of those on the notum. The gills have 6 tripennate leaves with greyish lamellae. The rachis of the leaves is dark at the base of its external face and white inside. In the specimen found in this study, we observed about 8–10 lamellae on each rhinophore, but it was not possible to define the number of gill leaves. Moreover, the specimen reported by us had very similar external morphological features to those of the specimens figured in Wirtz (1999, Plate 4, fig. 6) and in Trainito & Doneddu (2014: 70). Therefore, on the basis of specimens reported in the mentioned literature, we believe that the specimen found by us was *A. millegrana*.

Just like to Dayrat (2010), Trainito & Doneddu (2014) and Ballesteros et al. (2016), we believe that within the genus *Taringa* (which previously included *A. millegrana*) there are a lot of morphological similarities among species, thus further anatomical and molecular studies are necessary to clarify species delimitation inside this group.
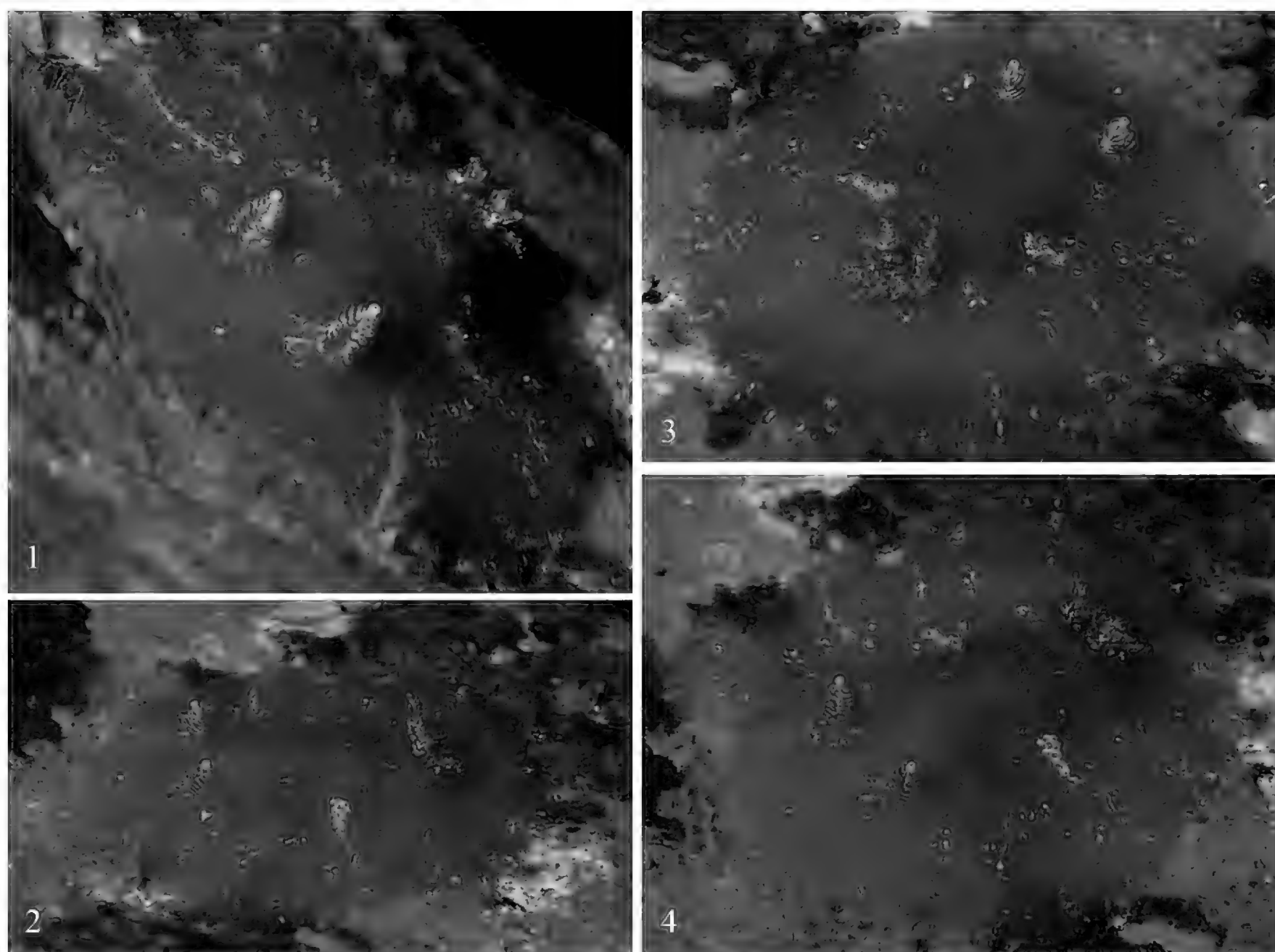
Figures 1–4: *Aporodoris millegrana* specimen found in Santa Maria La Scala (Sicily). Fig. 1) Anterior view showing the pattern of tubercles on the notum. Fig. 2) Right lateral view of the individual. Fig. 3) Posterior view of the specimen. Fig. 4) Dorsal view of the specimen (photos A. Lombardo).

CONCLUSIONS

In this study, the presence of *A. millegrana* in the Ionian Sea and in the central Mediterranean Sea was reported for the first time. Indeed, this species has been previously reported only in the Atlantic Ocean and in the western Mediterranean Sea. In the last years, some Atlantic Nudiplera, which were never reported before, have been increasingly found in the Ionian Sea. For example, *Pleurobranchus wirtzi* Ortea, Moro & Caballer, 2014, distributed in Canary Island, Madeira and Azores, and *Taringa tritorquis* Ortea, Perez & Llera, 1982, distributed in Canary Islands and presumably Madeira, have been recently reported for the first time in the Mediterranean Sea (Lombardo & Marletta, 2019; Gerovasileiou et al., 2020; Lombardo & Marletta, 2020). It has been observed that many Atlantic marine species introduced in the Mediterranean Sea, succeed in spreading eastwards within the Basin. Therefore, it has been hypothesized that the dispersal of Atlantic species and their propagules is driven by the hydrodynamic regime related to the Atlantic current in the western Mediterranean Sea. Moreover, the colonization success of Atlantic species into the Mediterranean Sea is related to the climate match between their native and their colonized environment. Indeed, a species expanding into an area within the same latitudinal range can experience similar environmental conditions (e.g., temperature) and successfully establish in the new area Lasram et al., 2009). In view of the rising water temperature in the Mediterranean Sea, the Atlantic termophilic species, like *A. millegrana*, *P. witzi* and *T. tritorquis*, could settle in new areas of the Mediterranean Sea. Nevertheless, it is not excluded that maybe these species, since are rare and with a sciaphilous behaviour, could be underestimated until now in the Mediterranean Sea.

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Structure of buffaloes *Syncerus caffer* (Sparrman, 1779) (Mammalia Bovidae) of the Comoe National Park (North-East Ivory Coast)

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ABSTRACT   This study was conducted from January to August 2018 in Comoe National Park (CNP), Ivory Coast. This park is home to a great biological diversity in general and an important fauna of large mammals in particular. It is one of the priority areas of the West African protected areas network. Today, many wildlife species are threatened by intensive poaching. Despite the importance of this scourge, the information available on buffaloes in CNP is still very patchy. In order to determine some parameters of the conservation status of buffalo populations, this study was conducted in the central zone and southern zone of CNP. The camera-trap method (12 camera-traps) made it possible to determine the different age classes and sex ratios of the buffalo populations. Family units were observed 11 times on all two (2) sites corresponding to 34.38 % of the events (32). The average size of family units is 23.18 individuals (standard deviation = 25.52; N = 11) with maximum of 77 individuals. Eleven (11) different groups (herds) of 255 individuals were identified with an average size of 32 individuals per herd. In the center, four groups composed of 15 adults, 31 subadults, 37 juveniles and seven calves were identified. In the south, seven groups with 34 adults, 49 subadults, 79 juveniles and three calves were observed. Calves were more numerous in the herds observed in the center than those identified toward the periphery at the 5 % (P-value = 0.0254). This high presence of calves in the herds observed in the center would reflect the fact that the buffaloes retire to the central part of the park for calving. Based on the high proportion of individuals in the young age classes (juveniles 44.5 % and subadults 32.07 %) and the sex ratio 1.65 (one male for 1.65 females), the buffaloes population in the CNP was considered viable. However, the inbreeding rate and anthropogenic pressures have yet to be evaluated to complete these data.

KEY WORDS   Buffalo; Structure; Population; Sex ratio; Comoe National Park.

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INTRODUCTION

Management of wildlife and wildlife habitats requires fairly accurate up-to-date knowledge of the population structure, including abundance social life, age classes and sex, which are fundamental demographic variables for the analysis of wildlife characteristics (Sinsin et al., 2006). In addition, large mammals have been the subject of numerous studies and well-established conservation plans

(Kassé et al., 2006; Kadjo et al., 2014). However, due to habitat loss, the vast majority of these mammals are confined to protected areas. But between 1970 and 2005, the overall number of African large mammals in protected areas fell by about 60 % on a continental scale and collapsed by about 85 % in West Africa (Craigie et al., 2010). Located in the north-east of the country, the Comoe National Park (CNP), Ivory Coast, is home to a high biological diversity in general and an important large mammals fauna in particular. This park is one of the priority areas of the West African protected area network (N'Goran et al., 2010; Kouakou et al., 2014; Bouché, 2016). Today, many wildlife species in this park are still threatened by intensive poaching (Djafarou & José, 2013; Koueita et al., 2018). Among these species is the buffalo for which the available information is still very patchy. However, the buffalo has a proven ecological role on vegetation, especially on the regeneration of higher plants (De Vos & Bengis, 1994). It is a species of high tourist value because it is part of the Big five game (Caro & Riggio, 2014). CNP, which is relatively well conserved and enjoys various national and international protection status, provides a favourable framework for studying the biology and ecology and defining the conservation status of this large wild West Africa ungulate, the buffalo. This study could also be benefit other wildlife taxa in CNP. The aim of this study is to determine some parameters of the conservation status of the buffalo populations. It will specifically (i) determine the structure of buffalo populations and (ii) the sex ratio of the observed groups in order to get an idea of the viability of the buffaloes in this park.

MATERIAL AND METHODS

Study area

Comoe National Park (CNP) is located in the north-east of Ivory Coast, between latitudes 8°30 - 9°37 north and longitudes 3°07 - 4°26 west and covers an area of 1,148,756 hectares (Fig. 1). It is crossed from north to south by the Comoe river in its western part. The climate of the CNP is the sub-humid tropical type with two season : a long rainy season and a long dry season. The dry season is well marked and can last up to 8 months from October to May. The rainy season is from June to September. March is the hottest month with temperatures around 37 °C while January is the least hot with an average temperature of around 15 °C. Annual rainfall is between 900 mm and 1200 mm with an average of 1084 mm per year (Fisher et al., 2002). The mean annual temperature varies from 26 °C to 27 °C (OIPR, 2015).

In the CNP, there is a wide variety of microclimates in relation to the observations made for certain habitats. There are four main types of vegetation: gallery forests, forest islands, wooded savannah and shrubby savannah.

Savannah formations occupy more than 80 % of the total park area and are therefore characteristic of the landscape of CNP (Schweter, 2016).

Sampling

The images from the trap cameras were classified by date and camera (Kely et al., 2019). The videos containing the images of the buffalo were used to determine the population structure of these animals. Camera traps that detected at least one buffalo were defined as positive camera traps, while those that did not detect buffaloes were defined as negative camera traps (Doré et al., 2011). For the counting of buffaloes on the videos, these animals are classified by sex (male or female) and by age class (adult, subadult, juvenile and calf). Age classes were determined based on horn shape and development, general morphology, changes in colour and coat texture (Sinclair & Grimsdell, 1978; Ndhlovu & Balakrishnan, 1991; Aberham
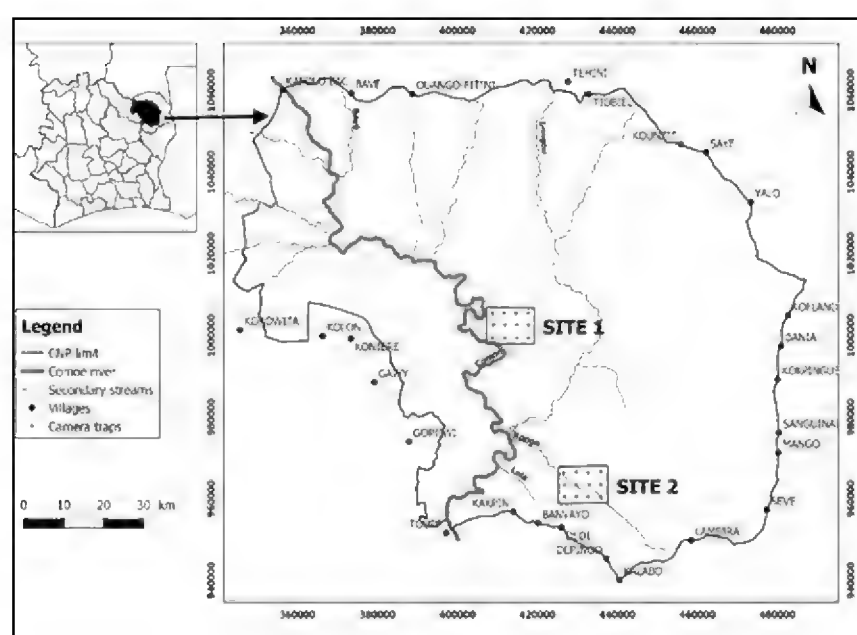

Figure 1. Map of the location and layout of camera traps in Comoe National Park, Ivory Coast.

et al., 2012). Four different age classes were therefore considered in this study (Table 1).

The methodology used in the study of the structure of buffalo populations in the CNP consisted of photographic trapping with the "Bushnell HD Trophy Cam Agressif Brown 119776" trap camera model. The camera traps were carried out in the central and southern zones of the CNP (Figure 1). These two zones were selected in areas with easy access and high concentrations of buffalo observed during the last aerial overflight in 2016 (Bouché, 2016). The two sites are 30 km apart.

Since the minimum home range of buffalo is estimated at 18 km² (Prins, 1996), a 3 km x 3 km grid covering an area of 144 km² was superimposed on the areas delimited for the study. Twelve (12) camera traps were placed, one inside each grid box. The choice of camera locations within the grids was dictated by buffalo presence clues (droppings, hoof prints, feeding track, etc.). The same cameras were used in each of the circumscribed areas (center and south) at different times. Data collection was carried out in two phases, from 3 January to 31 March 2018, in the central zone and from 2 June to 30 August 2018, in the southern zone. The camera traps that were removed after the first trapping phase were reused during the second phase. Each camera-trap was attached to a tree at a height of about 1 m

based on the average size of the buffalo (Nakashima, 2015; Hongo et al., 2016). The camera traps were set up in to video (hybrid) mode with recording sequences of one minute (60 s) each and separated by 30 seconds of intervals. The resolution of the photographs has been minimized (5 Mpx) to allow the memory cards to record more data. Information obtained from separate video sequence of less than 30 minutes are considered to information on the same buffalo herd (O'Brien et al., 2003; Nakashima, 2015; Hongo et al., 2016). Access to the navigation point of each camera trap was by navigation using GPS and compass. Once at the point indicated for the installation of a specific camera trap (theoretical position), we prospected within a radius of 200 m to choose the most appropriate position (Ancrenaz et al., 2012), in order to optimise buffalo detection (actual position). This actual position was chosen on the basic of the presence of a waterhole or buffalo signs (Ancrenaz et al., 2012; Mermod, 2012; Hedwig et al., 2018). The camera trap was installed to avoid direct exposure to the sun. It was oriented so as to avoid having seedlings or obstacles in the field of view. The camera traps were visited once a month during the trapping period to change batteries and SD memory cards.

After grouping the detections into events, we first counted the number of individuals for each

AGE GROUPS	PHYSICAL CHARACTERISTIC	REFERENCES
Calf (< 12 months)	They may or may not have visible horn buds, short, clearly visible horns, V-shaped upwardly protruding horns or straight horns growing outwardly and rearwardly. The coat of these animals is dark olive brown to black and appears smooth and soft, lighter to chocolate brown or yellow brown.	Funston, 1992; Pienaar, 1969
Juvenile (13 - 25 months)	This 13 to 24 months old age class of buffalo consist of individuals that are about twice as long as the horns. The horns are slightly curved outward, but are still widely separated at the tips. The coat is darker, rougher and brown.	Funston, 1992; Pienaar, 1969
Subadult (25 - 48 months)	These are buffaloes between 25 to 48 months old. They have sweeping horns on the inside. Male individuals have a huge frontal mass that covers the entire top of the skull above the eyes. The coat is uniformly smooth and dark chocolate brown in colour and turns black in females.	Sinclair, 1977; Pienaar, 1969
Adult (> 48 months)	Adult buffaloes are those over 48 months old. These animals considered adult have broad, back-sweeping horn tips. The colour of the coat becomes black.	Pienaar, 1969

Table 1. Characteristics of buffalo age classes.

event. This number is considered the size of the observed group (Bezerra et al., 2014; Hedwig et al., 2018; McCarthy et al., 2018). We then calculated the average size of buffalo groups in CNP, with or without the solitary individuals (Merz, 1986 ; White et al., 1993; Turkalo et al., 1996 ; Vanleeuwe et al., 1997; Maréchal et al., 1998; Querouil et al., 1999; Theuerkauf et al., 2000). Groups consisting of two or more individuals have been considered as "family units" (White et al., 1993; Maréchal et al., 1998).

The trapping effort is expressed in camera days and represents the number of days a camera is in operation multiplied by the corresponding number of cameras.

The catch rate corresponds to the number of independent photographs (useful catches) for the whole system reduced to a catch effort in terms of "nights-traps". A capture is useful when the information obtained from the video sequences is less than 30 minutes apart. The number of identified buffalo individuals is determined by counting individuals in each video sequence (useful capture). The correspondence between the images and the number of buffalo individuals was made through the dates of capture.

The sex ratio represents the ratio of the number of males and females overall and in each age group.

The chi² test was used to compared the abundance of buffalo by age class between the two zones. The student's t-test allowed to compare the composition of the buffalo populations observed in the center and south of the park. Finally, the non-parametric Kolmogorov-Smirnov test allowed to compare the distribution of buffaloes in the center and south of the park. The different tests were carried out with the software Xlstat 2016 version 18.02.01 and STATISTICA version 10.0.1011.7.

RESULTS

At the end of the two trapping phases, the data from 24 photographic traps were taken into account in our analysis. Twelve photographic traps in the central part and 12 photographic traps in southern part. All photographic traps functioned normally during both trapping phases. Of the 12 camera traps in the central part of the park, 7 were positive for the presence of buffalo (Fig. 2). In the south of the park, of the 12 photographic traps, 10 were positive for the presence of buffaloes (Fig. 2).

In the center of the park, 14 buffaloes captures were obtained, 12 videos were useful for the identification of individuals, which allowed the detection of 90 individuals. In addition, the trapping effort was 332 camera days (Table 2). In the southern part of the park, 21 buffaloes captures were obtained, 20 videos were useful for the identification of individuals, which allowed the detection of 165 individuals. In addition, the trapping effort was 345 camera days (Table 2).

Family units (group size greater than or equal to two) were observed 11 times at both (2) sites, corresponding to 34.38 % of the events (32). Considering the number of individuals observed (255), we deduce that 96.08 % of individuals live in a family unit, while 3.92 % of individuals live alone (N = 255). For family units, the observed modal size is two (2) individuals, with three (3) events, representing 27.27 % (N = 11) of the events. The average size of family units is 23.18 individuals (standard deviation = 25, 52; N = 11) with a maximum of 77 individuals.

Eleven (11) different groups (herds) of 255 individuals were identified with an average number of 32 individuals per herd. In the center, four groups composed of 15 adults representing 29.7 % (N = 90) of the population, 31 subadults representing 47.88 % (N = 90) of the population, 37 juveniles representing 20.6 % (N = 90) of the population and seven calves representing 1.82 % (N = 90) of the population were identified. While in the south, seven groups with 34 adults representing 34.44 % (N = 165) of the population, 49 subadults representing 41.11 % (N = 165) of the population, 79 juveniles representing 16, 67 % (N = 165) of the population and three calves representing 7.78 % (N = 165) of the population were observed. All solitary individuals observed were adults. Considering family units only, we have 3.27 % (N = 245) calves, 45.71 % (N = 245) juveniles, 31.84 % (N = 245) subadults and 19.18 % (N = 245) adults. Only four family units (36.36 %; N = 11) are made up of adults only, all of which are groups of two individuals. The remaining family units (63.64 % ; N = 11) are mixed, consisting of calves, juveniles, subadults and adults. The non-parametric Kolmogorov-Smirnov test showed that the distribution of buffalo by age group in the center and south is the same (P = 0.627) (Fig. 3).

In the central part of the park, proportion analysis showed that, according to t-test, there are more juveniles (P = 0.0001) and subadults (P = 0.0001)

Figure 2. Buffaloes observed in the CNP in the central zone (left) and in the southern zone (right).
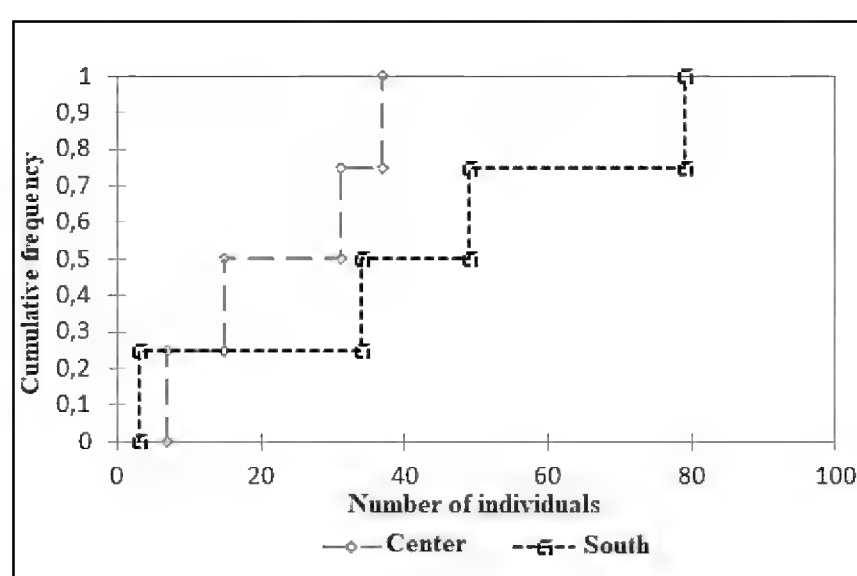

Figure 3. Distribution of buffalo by age group in the center and southern CNP.
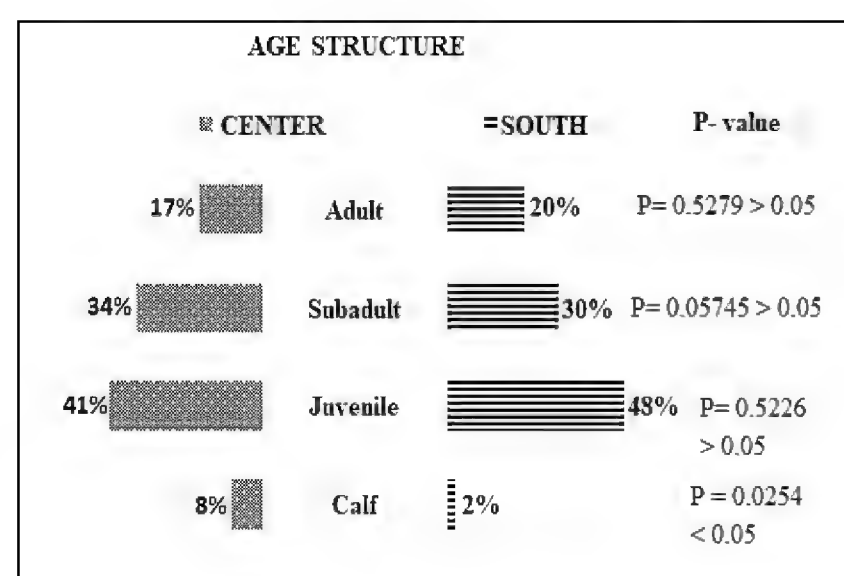

Figure 4. Composition of buffalo populations by age class in central and southern CNP.

than calves. However, no significant difference was observed between adults and calves (t-test, P = 0.108). Comparison of the proportions of juveniles and subadults showed that there was no significant difference between the proportions observed in these two age groups (t-test, P = 0.441). On the other hand, more juveniles than subadults were observed in the central part (t-test, P = 0.000). Finally, the number of subadults is significantly different from that of adults (t-test, P = 0.009).

In the southern zone of the park, a significant difference was observed between the number of calves and the other age classes, particularly juveniles (P = 0.0001), subadults (P = 0.0001) and adults (P = 0.0001) (Fig. 4). Proportions analysis showed that there are more juveniles than subaduls (t-test, P = 0.001) and adults (t-test, P = 0.0001). No significant difference was observed between the number of subadults and adults (t-test, P = 0.074). There

was also significant difference in the class composition of infants by zones.

Males and females, with average numbers of 22 and 41.75 individuals, account 35 % and 65 % respectively, resulting in an average sex ratio of 1 male to 1.65 females (Table 3). The proportion of females having young is 35.71 %. Based on the high proportion of individuals in the young age classes (juveniles 44.5 % and subadults 32.07 %) and the sex ratio 1.65 (1 male for 1.65 females), the buffalo population in CNP was considered viable.

DISCUSSION

Many of the videos viewed did not show buffalo visits or other events that could have triggered the camera. These artefactual detections considered false positives accounted for 30 % of the recordings. The

major problem with these recordings is that they consume a lot of memory and battery life. In addition, they waste a considerable amount of time when processing the data. The videos viewed without recordings were caused by wind and vegetation movement in the detection field of the device. Hence, it is important clearing the vegetation in the aircraft's field in anticipation of the gusts of wind that will occur. Other causes of false positives may be suspected, such as an animal leaving the camera's field of detection before it is triggered, or an animal entering the camera's field of detection without entering the field of view. There has often been the problem of identifying individuals with certainty. Indeed, in some cases the animal could not be identified because it was not completely in the field of view of the camera. In other cases, the quality of the image, often black and white, did not make it possible to accurately determine the identity of the individual when he presented many similarities with other individuals (case of hartebeest with buffalo calves). The lack of identification also led to a loss of data.

The analysis of the social structure of buffalo in the CNP based photographic images made it possible to identify eleven (11) different groups (herds) of 255 individuals with an average of 32 individuals per herd. In the center, four groups composed of 15 adults, 31 subadults, 37 juveniles and seven calves were identified. In the south, seven groups with 34 adults, 49 subadults, 79 juveniles and three calves were observed. It is however remarkable that the most represented age classes were juveniles and subadults, which reflect a good dynamics of the buffalo population. This result is different from that of Megaze et al. (2012) where the age structure was dominated by adults who accounted for 72.6 % of the total population. Our results do not corroborate those of Natta et

al. (2014) where the buffalo population of the classified forest of Agoua located in central Benin appears to be ageing (90 % adults and subadult) against only 10 % of juveniles. The poor observation of calves is linked to the low mobility of these animals during their young age (0 to 5 months), the small size of these animals in relation to the height of the cameras (1 m from the cameras), the very small age range compared to the others (1 year instead of 2 years or more for the others) and the low number of adults (of reproductive age). Despite this difficulty of observation, the proportions of observations made in the two zones remain valid. The low observation of adults, which is a resource species, may be related to poaching. On the other hand, the high number of juveniles and subadults could be explained by the fact that these animals are even less sought after by poachers and are more fearful and therefore more vigilant. These animals will thus take the necessary precautions to move away from a human predator before the latter sees them. Juveniles and subadults, most of which are weaned, must feed and are therefore forced to travel in search of food, which would also reflect their high number. Calves were more numerous in the herds observed in the center than those identified towards the periphery. This high presence of calves in the herds observed in the center would reflect the fact that buffalo retire to the central part of the park for calving. Furthmermore, we obtain an average group size excluding solitary individuals of 23.18 individuals, which is much higher than the value of 15.98 recorded by Megaze et al. (2012).

The male to female sex ratio is one male for 1. 65 female. This result is different from that of Marzanne (2007) where the male / female sex ratio of buffaloes in the eastern Kalahari region of South Africa is two males to one female.

Sites	Trapping effort (camera days)	Number of buffalo catches	Useful captures	Number of buffalo individuals identified
Center	332 units	14	12	90
South	345 units	21	20	165
Average	338.5 units	17.5	16	127.5
Standard deviation	± 9.19	± 4.95	± 5.66	± 53.03

Table 2. Number of photographic catches and individuals of buffalo by area.

Age group	Sex		Sex ratio
	Male	Female	
Calf (< 12 months)	6	4	0.66
Juvenile [13 - 25 months [	39	77	1.98
Subadult [25 - 48 months]	22	58	2.64
Adult (> 48 months)	21	28	1.33
Average	22	41.75	1.65
Standard deviation	± 13.49	± 32.25	± 0.85

Table 3. Sex ratio of buffalo catches in the central and southern CNP.

CONCLUSIONS

The present study constitutes a first scientific database on the population structure of buffalo in Comoe National Park. It made it possible to describe the different age classes of the buffalo populations observed in the south and center of the park. The average size of family units is 23.18 individuals with a maximum number of 77 individuals. Eleven different groups of 255 individuals were identified with an average herd size of 32 individuals. The different groups observed in the central and southern zones are composed of calves, juveniles, subadults and adults. The age structure is dominated by juveniles, followed by subadults, adults and then calves. The male / female sex ratio is one male for 1.65 female. The buffalo population in the center and south of the park is considered viable. It is capable of sustaining itself in the long term. Therefore, subsequent conservation actions must be carried out in relation to the riparian populations in order to maintain this population. However, the inbreeding rate and anthropogenic pressures have yet to be evaluated to complete these data.

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Tridax procumbens L. (Asterales Asteraceae), a new record to the flora of the United Arab Emirates

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ABSTRACT   *Tridax procumbens* L. (1753)*, recently collected from the mountain area in Masafi of Fujairah emirate, is recorded as a new alien plant for the flora of United Arab Emirates (UAE). With this discovery, *Tridax* is also added as a new genus to the country's flora. *T. procumbens* is a weed that is native to the tropical and subtropical Americas and naturalized in many other countries as it can grow under a wide range of environmental conditions. This plant is locally of considerable medicinal importance in many countries as it can be used for the treatment of many diseases. Description, distribution, and photographs of this taxa, along with a distribution map, is presented. Herbarium and seed specimens of *T. procumbens* were deposited in the Sharjah Seed Bank and Herbarium (SSBH) of Sharjah Research Academy, UAE. The way of introduction of *T. procumbens* and time of its arrival in the UAE remains unknown. A more detailed study of the extent of the range of this species, its densities, and regeneration potential is suggested in the near future. The reports of new plant records to the flora highlight the importance of thorough botanical exploration in the country. Such botanical recording would be beneficial to nature conservationists and researchers alike.

KEY WORDS   Medicinal; new record; *Tridax procumbens*; United Arab Emirates; weed.

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INTRODUCTION

The Asteraceae (Compositae) is one of the largest vascular plant families, comprising about 2250 genera and 25000 species worldwide (Funk et al., 2005; Funk et al., 2009; Stevens, 2017). In the vascular flora of the United Arab Emirates (UAE), Asteraceae is the second largest family after Poaceae, with approximately 84 species "*includes both native and alien taxa*" and it is distributed in every habitat of UAE (Jongbloed, 2003; Karim & Fawzi, 2007; Shahid & Rao, 2015; SSBH unpublished database).

The genus *Tridax* L. is distributed mainly in Central and South Americas and more concentrated in Mexico. This genus includes about 26 species, which are native in the new world. One weedy species of this genus, *Tridax procumbens* L. (1753), is widely naturalized in the Old World. *Tridax* plants are annual, perennial, erect, procumbent habits (Powell, 1965; Holm et al., 1997), and occupy a variety of habitats.

With the increasing international trade, the number of accidental and intentional exotic introductions species is probably increasing (Keane & Crawley, 2002; Lehan et al., 2013). Like other oil-producing countries, UAE is rapidly developing, with more commercial and agricultural exchange. As a result of that, the possibility of new or alien

plants appearing in the flora of the country is very high. The UAE has a hyper-arid climate and harsh environmental conditions and limited resources (Sherif et al., 2009; Shabana et al., 2018). Therefore, the existence of any alien plant might have a negative effect on plant diversity and biodiversity of the UAE.

This article reports for the first time the existence of *Tridax procumbens* in the natural flora of UAE and provides some data about habitat distribution and current information on the population of the species.

MATERIAL AND METHODS

During field explorations of Sharjah Seed Bank and Herbarium (SSBH) in April 2020, the authors collected specimens and fruits of a plant belonging to family Asteraceae from the Masafi region of Fujjarih emirate. Along with plant samples, all relevant field data, including geographic coordinates of the collection sites, associated species, phenology, number of individuals, and habitats were recorded based on personal observations. Also, some morphological traits such as plant height and width, size (length) of 50 fruits (achenes and pappus), and 50 seeds without pappus were recorded.

Detailed morphological characters of plants were noted in the field and from herbarium material and then compared with the taxonomic information from relevant literature (e.g., Powell, 1965; Keil et al., 1987; Holm et al., 1997; Funk et al., 2005; Panda & Behera, 2019). Reference voucher specimens were deposited in the herbarium of SSBH, Sharjah, UAE.

Study area

The United Arab Emirates (Fig. 1) is located in the south-eastern part of the Arabian Peninsula between latitudes 22°40' and 26°00' north and longitudes 51°00' and 56°00'east. Its climate is hyper-arid with high temperatures that can reach up to 47 °C in August. The rainfall is very scarce and erratic, with around 110 mm per year (Böer, 1997; Sherif et al., 2009; Merabtene et al., 2016).

RESULTS AND DISCUSSION

Based on the detailed study of morphological characteristics and consultation of relevant literature, our observations allowed us to deduce that the collected plant specimens belong to *Tridax procumbens*. Accordingly, we conclude that the plant is *T. procumbens* (Figs. 2–7), a new record to the flora of UAE.

The flora of UAE (Karim & Fawzi, 2007) and the flora of the Arabian Peninsula and Socotra Island (Miller & Cope, 1996), as well as other available literature (e.g., Jongbloed, 2003; Feulner, 2011), were thoroughly checked, and no previous records of this plant was found for the UAE. Hence, we record the discovery of *T. procumbens* as new species and genera record to the flora of UAE. The identification of *T. procumbens* was made by the authors based on the morphological characteristics of plant and field experience and consultation of published accounts of *Tridax* (e.g., Powell, 1965; Holm et al., 1997). Prof. Mats Thulin and Prof. Wafaa Taiaa confirmed the identity of this species from the author's photographs. The way of introduction of *T. procumbens* and time of its arrival in the UAE remains unknown.

Tridax procumbens L. (1753) (Figs. 2–7)

SYNONYMS. *Amellus pedunculatus* Ortega ex Willd., *Balbisia elongata* Willd., *Chrysanthemum procumbens* (L.) Sessé & Moc., *Tridax procumbens* var. *canescens* (Rich. ex Pers.) DC. and *Tridax procumbens* var. *ovatifolia* B.L.Rob. & Greenm.

COMMON NAMES. Coat buttons and *Tridax* daisy.

DESCRIPTION. *Tridax procumbens* is a hairy, semi-prostrate perennial herb with woody base; 30 cm–50 cm high; with adventitious roots at nodes. Plant stems are procumbent, branched near the base, round, sparsely to very hairy. Leaves are simple, opposite, lanceolate to ovate, 2 cm–7 cm long and 1 cm–4 cm wide, with irregularly toothed margins, base wedge-shaped, shortly-petioled, densely hairy on both surfaces. Inflorescence is a terminal involucrate flower head "capitulum", solitary on erect peduncle 10 cm to 25 cm long. Involucre 2–3–seriate, ovate, acute to shortly acuminate, 5 mm to 6 mm long. Receptacle with oblong, hairy scales. Its ray flowers are few, 3–dentate, pale yellow, and disk flowers are tubular, 5–dentate, yellow to brownish-yellow, with recurved hairy segments. Fruit is a black achene covered with fine, pale hairs,

grayish-brown, base narrow, 2 mm long, 1 mm wide at apex, pappus in one row, unequal in length, 5 mm–6 mm long (Powell, 1965; Ivens, 1967; Holm et al., 1997; Graves, 2000). Interestingly, one individual can produce about 500 to 2500 fruits. Mostly, the plant is a perennial, but some refer to it as an annual or short-lived perennial (Holm et al., 1997).

DISTRIBUTION. *Tridax procumbens* is native throughout the tropical and subtropical Americas. Now its distribution is increasing in other parts of the world as naturalized species and found in about 60 countries such as Angola, India, Vietnam, Thailand, and Australia (Powell, 1965; Holm et al., 1997; NGRP, 2018; United States Department of Agriculture, 2018). *Tridax procumbens* is a weed widespread in many countries due to its ability to adapt to diverse environments and rapidly colonize new areas as well as its abundant seed production (Powell, 1965; Holm et al., 1997). It has agricultural impact as it is a weed of 31 crops (e.g., rice and wheat) in 60 countries (Holm et al., 1997). This plant has high seed germination (ca, 98%) and viability of seeds extending to 450 days. Its seed germination is high in light and wide temperatures and
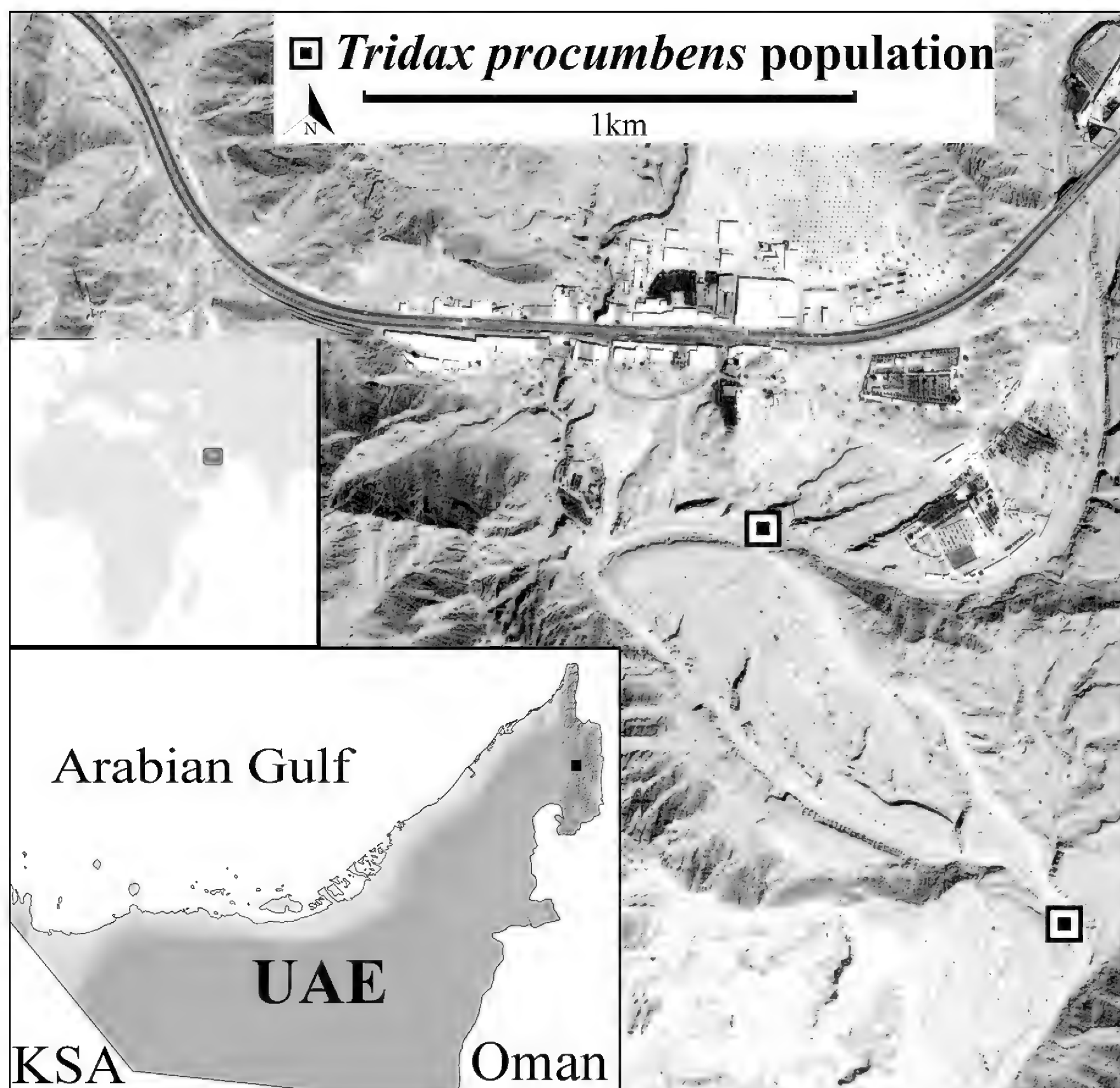
Figure 1. Map showing the distribution of records for *Tridax procumbens* in UAE.

pH range (Chauhan & Johnson, 2008; Vanijajiva, 2014). It is considered invasive in some countries, for example, China (Weber et al., 2008), Taiwan (Wu et al., 2004), and India (Panda & Behera, 2019). In the Arabian Peninsula, it is recorded from Saudi Arabia (Alfarhan et al., 1997; Thomas et al., 2016), Oman (Pickering & Patzelt, 2008; Patzelt et al., 2014), and Yemen (Hall et al., 2008; Hall et al., 2009).

In United Arab Emirates, *T. procumbens* was recorded from two sites in the south of Masafi, Fujairah emirate. The distance between the two sites is about one Km., (N: 25.282151, E: 56.121951, Alt.: 236 m and N: 25.288572, E: 56.116737, Alt: 338 m) (Fig. 1).

As this species has been recorded in three countries in the Arabian Peninsula, two from them are neighboring UAE (Hall et al., 2009; Patzelt, 2014; Thomas et al., 2016). Therefore, there is a possibility that the plant may transfer from the neighboring regions to UAE. However, we cannot confirm this, because at present there is not sufficient information available about the plant in these countries, such as its distribution, rate of spread, and its population size.

HABITAT AND ECOLOGY IN UAE. *Tridax procumbens* was found growing in mountain wadi banks and adjacent slopes at about 340 m altitude. In UAE, the mountain area contains a reasonable amount of water from rain compared to other habitats. This plant was associated with 19 species from 15 families, and the more dominant species were *Frankenia pulverulenta* L. (1753), *Aizoon canariense* L. (1753), *Cenchrus ciliaris* L. (1854), *Tephrosia apollinea* (Delile) DC. (1822), *Amaranthus viridis* L. (1763), *Cleome noeana* Boiss. & Popov (1916), *Andrachne aspera* Spreng. (1826), *Boerhavia elegans* Choisy (1849), *Datura stramonium* L. (1938) and *Citrullus colocynthis* (L.) Schrad (1838).

NUMBER OF INDIVIDUALS IN UAE. During the course of the survey, ca. 55 individuals of this species were recorded from the two sites. These can be categorized into mature, vegetative, and seedling stages. The mature individuals were five, two of them had both flowers and fruits, and the other three with flowers only. The other 50 individuals were recorded in the seedling and vegetative stage.

PHENOLOGY IN UAE. Flowering and fruiting of the plants have been seen in April–May. Recording mature individuals with flowers and fruits gives the impression that this species might be flowering and fruiting throughout the year. Observations on young individuals of this species suggest that the seedling emergence began before one week in the natural population (Figs. 2–7). Therefore, we recommend that this plant needs more monitoring in the near future to understand its phenological aspects in UAE.

PLANT AND FRUIT SIZE. The mean and standard deviation of plant height and width of the mature plants were (40.0 cm ± 7.9 and 86.0 cm ± 39.3, respectively). For small plants (vegetative and seedling), it was (4.5 cm ± 1.0 and 6.2 cm ± 1.2, respectively).

Mean and standard deviation of fruit size (length) was 0.5 cm ±. 0.1, the seed (achene) without pappus was 0.2 cm ±. 0.1 (Figs. 6, 7).

DISPERSAL TRAITS. Literature suggests that *T. procumbens* might have different dispersal patterns. The fruits of this species are able to disperse over long distances by both wind and water with its pappus (Shaukat et al., 2005; Vanijajiva, 2014). Another strategy includes restricted dispersal by falling fruit clusters together around the mother plant and stored in the soil seed bank (Shaukat & Siddiqui, 2004; Shaukat et al., 2005). It has been suggested that the production of numerous fruits that disperse by the wind for long-distance may make it invasive (Vanijajiva, 2014; Amutha et al., 2019).

In UAE, the distribution of *T. procumbens* in two sites indicate its possible long-distance dispersal as the fruits might have dispersed from one site to another while the small individuals around big plants express the restricted dispersal. The characteristics of long-distance dispersal, rapid growth and regeneration, and reproductive ability might enable the spread of *T. procumbens* in UAE.

USES OF *TRIDAX PROCUMBENS*. The plant is used as an ornamental, for feed and medicinal purposes, and its leaves are cooked as vegetables in some places (Prajapati, 2008; Acharya & Srivastava, 2010; Kethamakka & Deogade, 2014).

Tridax procumbens is used in a wide range in folklore medicine (Kethamakka & Deogade, 2014). It is used in the treatment of many ailments including diarrhea, malaria, stomach pains, diabetes, cough, fever in different countries in the world (Martín-Quintal et al., 2009; Ahirwar et al., 2010;

Figures 2–7. *Tridax procumbens* from United Arab Emirates. Fig. 2: whole plant "mature, vegetative, and seedling". Fig. 3: stem with leaves. Fig. 4: inflorescence "capitulum". Fig. 5: fruit set per head. Fig. 6: fruit clusters. Fig. 7: fruits size "achene and pappus".

Kethamakka & Deogade, 2014). In addition, many therapeutic activities such as anti-cancer, anti-bacterial, anti-fungal, antioxidant property, wound healing, and anti-diabetic are reported for this plant (Kethamakka & Deogade, 2014; Jhample et al., 2015; Saini et al., 2016).

In UAE, *T. procumbens* can be used as medicinal, ornamental, and for animal feed by keeping it under control to prevent its spread. Therefore, studying the spatial distribution of *T. procumbens* would help to understand the spread and predict its future distribution and invasion risk, as such information enables the development of means of invasion control.

CONCLUSIONS

Our findings contribute towards an update of the range of *T. procumbens*, a new alien plant record and a new genus added for the flora of UAE. Elsewhere this species is reported as a weed and also have invasive characteristics; therefore, regular field assessments should be undertaken to monitor the spread and population dynamics as well as observing any threats by it to native plants in UAE. Thus, it becomes more important to prevent future introduction of such alien species and their spread for safeguarding native plant diversity.

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On two rare species of Plio-Pleistocene marine molluscs of the Mediterranean Basin

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ABSTRACT   During the study of the Plio-Pleistocene malacofaunas of the Mediterranean Basin, the presence of *Liamorpha elegans* (de Folin, 1870) was ascertained. This species has never been previously reported in sediments of the lower-middle Pliocene of central Italy and was reported only once in sediments of the upper Pleistocene (Tyrrhenian sensu Auctores) of southern Italy. Further-more, the bivalve *Tugonia anatina* (Gmelin, 1791) was found in the Tyrrhenian of southern Spain. This is the second record in the upper Mediterranean Pleistocene after more than a hun-dred years; the specimen was compared with others, both from the Zanclean of the Guadalquivir Basin (Spain) and the Zanclean of Tuscany (Italy). Both species are discussed and illustrated.

KEY WORDS   Pliocene; Pleistocene; Pyramidellidae; Myidae

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INTRODUCTION

During research on the Plio-Pleistocene mala-cofaunas of the Mediterranean Basin, the presence of the species *Liamorpha elegans* (de Folin, 1870) was ascertained. This species was described in the past as belonging to the genus *Miralda* A. Adams, 1863. Its presence is confirmed in the upper Pleis-tocene of southern Italy, with new findings, and is also reported for the first time in the lower-middle Italian Pliocene. During the same research, speci-mens of the bivalve *Tugonia anatina* (Gmelin, 1791) were found in sediments of the Spanish upper Pleistocene, a species previously reported for this period, only once many years ago (Gignoux, 1913).

MATERIAL AND METHODS

The material examined, collected during surface research, comes from various Pliocene deposits, both in the Guadalquivir basin (Gonzales Delgado,

1985, 1988, 1989, 1993; Landau et al., 2011), and from the Zanclean/Piacenzian of central Tuscany (Brunetti & Della Bella 2006, 2008), the Tyrrhenian of Sicily (Ruggieri & Greco, 1965) and that of southern Spain (Torres et al., 2006). For the generic and suprageneric determinations we followed the WoRMS (2020).

ABBREVIATIONS AND ACRONYMS. H = maximum height of the shell, measured from the apex to the end of the siphon canal; L = maximum width of the valve; coll. = collection; ex = speci-men/s; CMB = M. Mauro Brunetti collection.

MATERIAL AND METHODS

Systematics

Classis GASTROPODA Cuvier, 1797
Superfamilia PYRAMIDELLOIDEA Gray, 1840
Familia PYRAMIDELLIDAE Gray, 1840

Genus *Liamorpha* Pilsbry, 1898
Type species *Lia decorata* de Folin, 1873

Liamorpha elegans (de Folin, 1870) - Figs. 1, 2

Mathilda elegans de Folin, 1870: 212–213, tab. 26,
        fig. 11
Pyrgulina sculptatissima Dautzenberg, 1910: 67,
        tab. 3, figg. 15–16
Miralda elegans (de Folin, 1870) - Hoenselaar &
        Moolenbeek, 1990: p. 65 figg. 1–5
Miralda elegans (de Folin, 1870) - Crovato & Mi-
        cali, 1990: p. 125, tab. 1, fig. 2
Chrysallida pulchra Jeffreys, 1874 - Gaglini, 1992:
        138, fig 146 (*nomen nudum*)
Liamorpha elegans (de Folin, 1870) - Aartsen, Git-
        tenberger & Goud, 1998: p. 9–11
Miralda elegans (de Folin, 1870) - Cossignani &
        Ardovini, 2011: p. 349 (figure not numbered)
Miralda elegans (de Folin, 1870) - Peñas, Rolan &
        Swinnen, 2014: p. 128, fig. 7 D–E

EXAMINED MATERIAL. Italy, Siena, Poggibonsi,
località Cipressino, 1 ex, lower-middle Pliocene.
Italy, Messina, Capo Milazzo, 2 ex, "Tirreniano".

REMARKS. For the discussion of this taxon at a
generic level, see Aartsen et al. (1998), with which
we agree. The characteristic sculpture of the teleo-
conca (Fig. 1A) prevents confusing the species with
any other, fossil and living. Despite this, Dautezn-
berg (1910) described it as a new *Pyrgulina sculp-
tatissima*, while admitting the strong resemblance
to de Folin's species: "*Cette petite espèce est remar-
quable par sa sculpture très saillante, qui lui donne
un aspect bien particulier. Il se pourrait qu'elle fût
la même que celle qui a été décrite et figurée par le
Marquis de Folin dans les Fonds de la Mer, I, p.
212, pi. XXVI, fig. 11, sous le nom de* Mathilda ele-
gans*: la sculpture est, en effet, fort semblable, mais,
par contre, il n'est pas fait mention, dans la de-
scription, du pli columellaire qui est bien visible sur
nos échantillons et qui fixe la classification de ce
Mollusque parmi les Odostomidés*".
        Even Gaglini (1992: 138), while describing
Chrysallida pulchra Jeffreys, 1874, a name unusable
in any case due to it being a nomen nudum, admitted:
"*Non si può fare a meno di notare la rassomiglianza
di questa specie con* Miralda elegans". However,
Liamorpha elegans features a quite variable teleo-
conch sculpture as specified also by Peñas et al.

(2014: 128): "*there are some differences between
populations in relations to the number of spiral cords
which are on the lower part of the last whorl and the
number of the axial ribs on its upper part*".
        Until now, the fossil finds of *Liamorpha elegans*
were limited to a single record for the Upper Pleis-
tocene of southern Italy (Crovato & Micali, 1990),
a presence confirmed in this contribution by the dis-
covery of two further specimens of the Tyrrhenian
of Capo Milazzo (Messina) (Fig.1B). *Liamorpha
elegans* is part of the typical warm fauna with Sene-
galese affinity described by various authors (Gig-
noux, 1913; Trevisan & Di Napoli, 1938;
Mirigliano, 1953; Segre, 1954; Malatesta, 1954;
Ruggieri & Buccheri, 1968; Settepassi, 1971; Rug-
gieri & Melone, 1975; Vazzana, 2008, Brunetti M.,
2018). The specimen of the lower-middle Tuscan
Pliocene was found in a characteristic sediment in
Petaloconchus sp. previously described by Brunetti
M. & Della Bella (2006, 2008). According to Peñas
et al. (2014), the species ranges from Guinea Bissau
to Angola, where it is frequently found, with ba-
thyal distribution from the infralittoral to the cir-
calittoral. So far there are only two reports for the
Mediterranean Sea (Hoenselaar & Moolenbeek,
1990, Formentera & Gaglini, 1991: Sicily) and for
Mauritania (van Aartsen et al., 1998; Cossignani &
Ardovini, 2011).

Classis BIVALVIA Linnaeus, 1758
Ordo MYIDA Stoliczka, 1870
Superfamilia MYOIDEA Lamarck, 1809
Familia MYIDAE Lamarck, 1809
Genus *Tugonia* Gray, 1842
Type species *Mya anatina* Gmelin, 1791

Tugonia anatina (Gmelin, 1791) - Figs. 3, 4

Mya anatina Gmelin, 1791: 3221
?*Tugonia anatina* (Gmelin, 1791) - Hörnes, 1870:
        32, tab. 6, figg 6a–b
?*Tugonia* (s. s.) *taltassei* Chavan, 1950: 31, fig. 1.
?*Tugonia* (*s. s.*) *sherborni* Glibert & van de Poel,
        1966: 37
?*Tugonia* (*s. s.*) *sherborni* Glibert & van de Poel,
        1971: 5, tab. 2, fig. 6.
Tugonia anatina (Gmelin, 1791) - Pantoli & Raffi,
        1981: 73, tab. 1, figg. 1–20.
?*Tugonia* (*Tugonia*) *anatina* (Gmelin, 1790) - San-
        tos & Mayoral, 207: 47, tab. 1, fig. 9.

Figures 1, 2. *Liamorpha elegans* (de Folin, 1870). Fig. 1: Cipressino (Poggibonsi, Siena, Italy) lower-middle Pliocene, H = 2.2 mm CMB X015. Fig. 2: Capo Milazzo (Messina, Italy), Tyrrhenian. H = 1.6 mm CMB X015A. Figures 3, 4. *Tugonia anatina* (Gmelin, 1791). Fig. 3: Roqueta de Mar (Almeria, Spagna), Tyrrhenian, right valve L = 25.2 mm CMBS726. Fig. 4: Santa Catalina (Huelva, Spain), Zanclean, right valve L = 23 mm CMBZ395.

Tugonia anatina (Gmelin, 1791) - Chirli, 2015: 132, tab. 30, figg. 5–7.

EXAMINED MATERIAL. Spain, Almeira, Roqueta de Mar, 1 ex, Tyrrhenian. Spain, Huelva, Santa Catalina, 20 ex, Zanclean. Italy, Siena, Poggibonsi, 5 ex.

REMARKS. This taxon too, due to the morphological characteristics of the valves, cannot be confused with other Plio-Pleistocene species. We were not able to examine current material, other than the material depicted in Pantoli & Raffi (1981). Thus we hypothesized that living and Plio-Pleistocene populations belong to the same species. The specimen found in the Spanish Upper Pleistocene (Fig. 3) does not show significant differences with other Italian and Spanish examples of the lower Pliocene (Fig. 4), except for a greater sturdiness of the shell and a radial sculpture with less close ribs. Still to be clarified, in my opinion, is the relationship be-tween the Miocene species *Tugonia taltassei* Chavan, 1950 from the Tortonian of Morocco and *Tugonia sherborni* Glibert & van de Poel, 1966 (Fig. 6), from the Portuguese upper Miocene, as well as the relationship with *Tugonia anatina* from Hörnes (1870) for the Austrian Miocene and from Santos & Mayoral (2007) for the Portuguese Miocene. Whether all these species are valid or correspond to *Tugonia ornata* (Basterot, 1825) (Figs. 7, 8) is not the purpose of this paper. Only the examination and direct comparison of a greater number of specimens could definitively clarify the exact systematic position of the taxa treated. The specimen found comes from a layer of coarse sand mixed with reddish gravel (Fig. 5), one of the few Pleistocenic deposits Roqueta del Mar (Almeria, Spain) that survived the reckless urbanisation in the area. On the same level, *Persistrombus latus* Gmelin, 1791 (= *Strombus bubonius* Lamarck, 1822) and *Conus ermineus* Born, 1778, (= *Conus testudinar-*
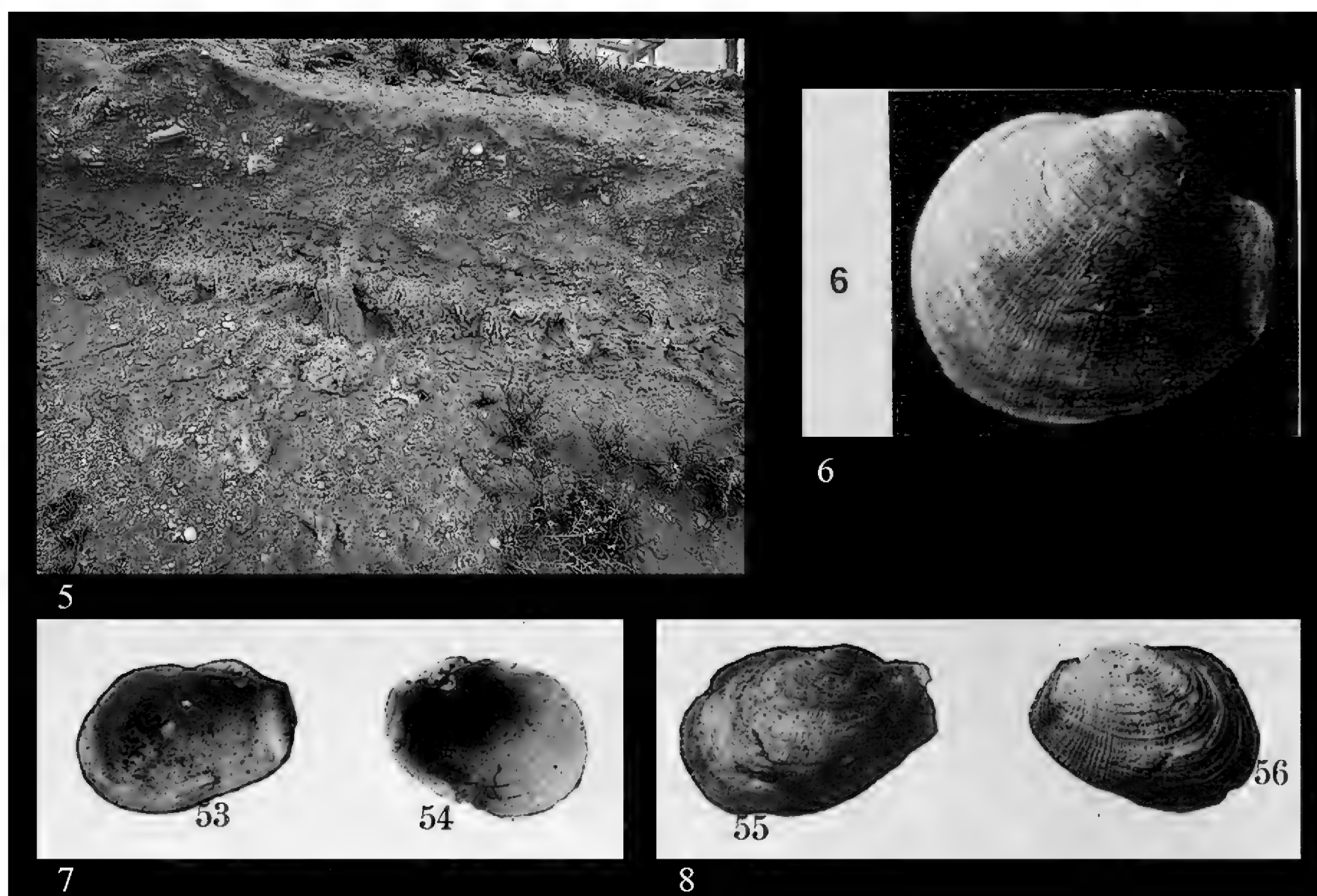
Figure 5. Geological level from which this specimen of *Tugonia anatina* comes. Figure 6. *Tugonia* (*s. s.*) *sherborni* Glibert & van de Poel, 1966, holotype, Adiça (Portogalo), Tortonian, left valve L = 20 mm, original figure rom Glibert & van de Poel, 1971. Figures 7, 8. *Tugonia ornata* (Basterot, 1825) St. Avit (France) lower-middle Miocene, left valve L = 20.2 mm, right valve 18.2 mm.

ius Hwass in Bruguière, 1792) have been found which allows us to date the valve of *Tugonia anatina* the Tyrrhenian period. The species had previously been reported only once by Gignoux (1913) in the Tyrrhenian of Monastir (Tunisia). This further report confirms that *Tugonia anatina* belongs to the well-known group of species with Senegalese affinity of the upper Pleistocene of the Mediterranean Basin.

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Brachyta (Fasciobrachyta) bureschi (Kantardjieva-Minkova, 1957): a distinct species from North East Bulgaria and South East Romania (North East Balkan Penisula) (Coleoptera Cerambycidae Lepturinae Rhagiini)

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ABSTRACT　　　*Brachyta (Fascibrachyta) bureschi* (Kantardjieva-Minkova, 1957) (Coleoptera Cerambycidae Lepturinae Rhagiini) is reevaluated as a distinct species. This taxon was described from Bulgaria (Varna and Kavarna localities) as a form. In Bulgaria, the species was recently found near the second locality of the original description - Vidno Vill., Kavarna municipality. For long time it was recognized as a synonym of *B. (Fascibrachyta) balcanica* (Hampe, 1871).

KEY WORDS　　　Bona species; Cerambycidae; Lepturinae; Rhagiini; Bulgaria; Romania.

INTRODUCTION

In the last few years several pictures of *Brachyta* sp. found in Romania appeared on the social media (www.entomologiitaliani.it, facebook) and questions marks were raised regarding the real identity of these specimens.

For Romania, the first mention of *Brachyta*, as *Brachyta balcanica*, appear in Fleck from Valul lui Traian (near Constanța) (Fleck, 1905) and later in the monographic work dedicated to Cerambycidae from Sergiu Panin and Nicolae Săvulescu were it is mentioned that all specimens from Romania belong to form *bureschi* Minkova (Panin & Săvulescu, 1961). In this book, one specimen from Bulgaria (Cavarna, VI.1925, leg. Lepşi I.) is mentioned as being in Dr. Karl Petri collection. A recent catalogue of Dr. Karl Petri collection, that is stored in Natural History Museum from Sibiu, fails to find this spec-

imen in collection (Tăuşan & Bucşa, 2010). Later, *Brachyta balcanica* was mentioned in the first part of the catalogue of Cerambycidae from "Grigore Antipa" National Museum of Natural History from Bucharest (Serafim, 2005).

In 2018, one of the authors (Cosmin Manci) visited the biotope in Dobrudja region (Cotu Văii (CT), Romania) and collected a small series of specimens. After a study of these specimens, previously identified as *Brachyta balcanica* (Hampe, 1871), it was clear that they belonged to a distinct species. Checking inside the synonyms (Löbl & Smetana, 2010, Kantardjieva-Minkova, 1957), we found out that a "form" of *Brachyta balcanica* was described from north-eastern Bulgaria (Varna and Kavarna localities). Subsequently, a series of specimens were collected from north-eastern Bulgaria (Vidno village, Kavarna municipality), by the last author. Two additional specimens, recently collected from

Bezhanovo Village (north-eastern Bulgaria near the Romanian border) have also been found in BFUS collection of Sofia University "St. Kliment Ohridski". After the comparison of the newly collected specimens with the original description and the syntype of *Evodinus balcanicus bureschi* (Kantardjieva-Minkova, 1957) from the NMNHS collection, we decided that the Romanian and Bulgarian populations from the Black Sea coast in the neighboring territory of the river Danube mouth (steppe vegetation) belong to *Brachyta* (*Fascibrachyta*) *bureschi* (Kantardjieva-Minkova, 1957). The status of this taxon is the object of the present paper.

ACRONYMS. BFUS: Zoological Collection of Sofia University "St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria. CAR: Adrian Ruicanescu, Cluj-Napoca (CJ), Romania, private collection. CCM: Cosmin Manci, Iasi (IS), Romania, private collection. CGS: Gianfranco Sama, Cesena (FC), Italy, private collection. CNHM: Croatian Natural History Museum, Zagreb, Croatia. CPR: Pierpaolo Rapuzzi, Cialla di Prepotto (UD), Italy, private collection. MGAB: "Grigore Antipa" National Museum of Natural History, Bucharest, Romania. NMNHS: National Museum of Natural History in Sofia, Sofia, Bulgaria.

RESULTS

Brachyta **(*Fasciobrachyta*) *bureschi*** (Kantardjieva-Minkova, 1957) *bona species* (Figs. 1, 2)

Evodinus balcanicus forma *bureschi* Kantardjieva-Minkova, 1957, Izvestiya na Zoologicheskiya Institut s Muzei 6: 542. Type loc.: Varna and Kavarna (Bulgaria).

EXAMINED MATERIAL (Fig. 18). *Brachyta* (*Faciobrachyta*) *bureschi* (Kantardjieva-Minkova, 1957). Bulgaria: 1 female: Kavarna, 15.IV.[19]09 (Lectotype); 1 male: Stalin [= Varna], Soluk Salaar, 24.V.1953, Karnoschitzky [lgt.]; 2 males: NE Bulgaria, Dobrich prov., Bezhanovo Vill., Kachamaka Place, 70 m., 43°43'23"N 28°24'25"E, 04.IV.2010, D. Kaynarov and S. Beshkov lgt. (BFUS); 7 males and 8 females: NE Bulgaria, Dobrich prov., Kavarna municipality, near Vidno Vill., 80 m., 43°34'01,5"N 28°24'21,4"E, 25.IV.2019, steppe vegetation near farmland, in flowers of *Paeonia tenuifolia* L., D. Gradinarov and B. Zlatkov lgt. (BFUS). 19 specimens.

The last locality (Vidno Vill.) is located less than 10 km from the type locality and appears to represent the same population of the species.

Romania: 1 female: Agigea, 9.VI.1958, F. Cârdei lgt. (MGAB); 1 male: Murfatlar, 17.V.1962, N. Săvulescu lgt. (MGAB); 2 females: Hagieni, 29.V.1963, N. Săvulescu lgt. (MGAB); 3 females: Hagieni, 21.V.1965, N. Săvulescu lgt. (MGAB); 1 male and 10 females: Hagieni, 29.IV.1991, N. Săvulescu lgt. (MGAB); 6 males and 7 females: Hagieni, 30.IV.1991, N. Săvulescu lgt. (MGAB); 2 males and 11 females: Hagieni, 1.V.1991, N. Săvulescu lgt. (MGAB); 7 males and 24 females: Cotu Văii, 19.IV.2018, C.-R. Stanciu lgt. (MGAB); 2 females: Hagieni, 6.V.1993, E. Nitu lgt. (CAR); 2 males and 1 female: Cotu Văii, 23.IV.2019, R. Georgescu lgt. (CAR); 10 males and 7 females: Cotu Văii, 19.IV.2018, C.-R. Stanciu lgt. (CCM); 3 females: Cotu Văii, 23.IV.2018, C-O. Manci lgt. (CCM); 3 males: Hagieni, 24.IV.2019, C-O. Manci lgt. (CCM); 16 males and 24 females: Cotu Văii, 25.IV.2019, C-O. Manci lgt. (CCM); 3 males and 1 female: Coroana, 26.IV.2019, C-O. Manci lgt. (CCM); 3 males and 2 females: Cotu Văii, 27.IV.2019, C-O. Manci lgt. (CCM); 3 males and 12 females: Coroana, 27.IV.2020, C.-R. Stanciu lgt. (CCM); 2 males and 1 female: Hagieni, 28.IV.2020, C.-R. Stanciu lgt. (CCM); 2 females: Coroana, 28.IV.2020, C.-R. Stanciu lgt. (CCM). 172 specimens.

Brachyta (*Fasciobrachyta*) *balcanica* (Hampe, 1871). Croatia: 1 male: Varaždin, Lepoglava, [1]933 (CNHM). 1 specimen. Serbia: 1 male and 1 female: Devojač bunar, Deliblato Peščara, 24.IV.2004, N. Ilić lgt.(CPR). 2 specimens. Albania: 1 female: Kula Ljums, 18-26.V.[19]18 (CGS). (1 specimen). Bulgaria: 1 male: Slivno, 1902 (CGS); 2 females: Stara-Zagora, Col N. Nedelkov (NMNHS); 2 males: Burgas, P. Tschorbadjieff [lgt.] (NMNHS); 1 male: Haskovo, V. 1897, Col. D. Ioakimov (NMNHS); 2 females: Burgas, IV.[1]906, Col N. Nedelkov (NMNHS); 1 male: Sliven, Kushbunar [Place], 27.V.[1]923, P. Tschorbadjieff [lgt.] (NMNHS); 1 male: Sakar Pl. [=Sakar Mts.], 26.V.1951, Dr. N. Atanassov and V. Petrov (NMNHS); 1 female: Sarnena Gora Mts., Beter Peak, 809 m., 42°30'41,2"N 25°37'46,4"E, 28.V.2018, mixed deciduous forest, D. Georgiev lgt. (BFUS); 1 male: W Rhodopes Mts., 2 km SW Asenovgrad, 780 m., 41°59'46,7"N 24°50'44,5"E, 26.IV.2017, mixed deciduous forest,
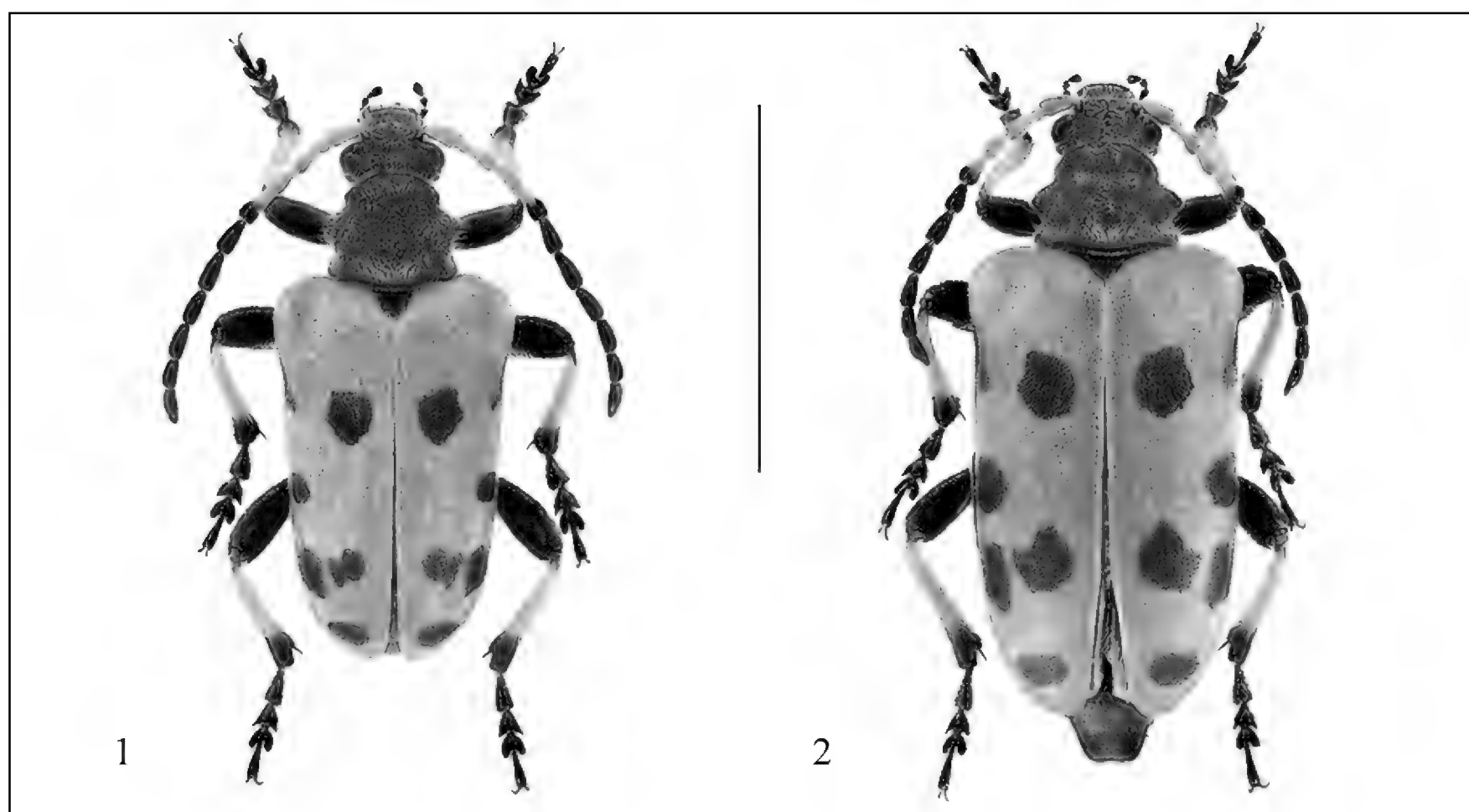
Figures 1, 2. *Brachyta bureschi* habitus: male (Fig. 1) and female (Fig. 2). Scale bar: 10 mm.

O. Sivilov and B. Zlatkov lgt. (BFUS); 5 males and 7 females: W Rhodopes Mts., 2 km SW Asenovgrad, 780 m., 41°59'46,7''N 24°50'44,5''E, 26.IV.2019, mixed deciduous forest, on leaves of *Paeonia peregrina* Mill., D. Gradinarov and B. Zlatkov lgt. (BFUS). 24 specimens. Greece: 1 male: Thrace: Evros, Ft. De Pesani, 30.IV.2004 (CPR); 61 males and 21 females: Thrace: Evros, Loutros Dadia, 29 Km S Dadia, 25–27.IV.2007 (CPR); 1 male: Thrace, Sapka Mts., Nea Santa env., 41°07'29,06''N 25°53'48,60''E, 30.IV.2014, D. Vit lgt. (CPR); 1 male and 1 female: Thrace: Panagiá, Pangaion Mt., 24.V.2016. I. Martinu lgt. (CPR); 3 males and 8 females: Thrace: Panagiá, Pangaion Mt., 16.V.2018, 970 m., I. and R. Martinu lgt. (CPR); 1 female: Thrace, Rodopi Mts., Nea Santa vill., 760 m., 7-8.V.2014. S. Dementyev lgt. (CPR); 1 male: Thrace: Panagiá, Pangaion Mt., 24.V.2016. I. Martinu lgt. (CCM); 1 female: Thrace: Panagiá, Pangaion Mt., 16.V.2018, 970 m., I. and R. Martinu lgt. (CCM). (100 specimens). Turkey: 1 female: Gümüşhane prov., Gümüşhane, VI.1972, C. Holzschuh lgt. (CPR); 1 female: Giresun prov., Soğanli, 2000 m., 25.VI.1992, A. Del Fabbro lgt. (CPR); 1 male and 1 female: Giresun prov., Soğanli, 2000 m., 25.VI.1992, G. Proscia lgt. (CPR); 1 female: Giresun prov., Soğanli, 1800 m., 25.VI.1987 (CPR); 3

males and 1 female: Erzincan prov., Caglayan, Girlevik Mts., 2100 m., 12–19.VI.1993, P. Kabátek lgt. (CPR); 1 male: Erzincan prov., 20 Km E Erzincan, Çakrilar, Munzur Dağ., 1800 m., 39°36'N 39°23'E, 15-16.VI.2005, T Rybárik lgt. (CPR); 2 males and 2 females: Sivas prov., North of Zara, Kurbagalibeli Geç., 1800 m., 4.VI.1998, S. Kadlec lgt. (CPR); 1 female: Sivas prov., North of Zara, Kurbagalibeli Geç., 1800 m., 3.VI.1998, M. Rejzek lgt. (CPR); 2 males and 2 fermales: Sivas prov., env. Zara, 3–5.VI.2003, D. Farbiak lgt. (CPR). 18 specimens.

Brachyta (*Fasciobrachyta*) *petriccionei* Rapuzzi, Bologna et Poloni, 2019. Italy: 1 female: Abruzzo, Monte Morrone (PE), 1500 m, 23.V.2019, R. Poloni, P. Rapuzzi, M.A. Bologna, M. Carafa and B. Petriccione lgt. (CPR); 1 male: Abruzzo, Monte Morrone (PE), 1500 m,, 29.V.2019, M. Carafa lgt., 2 exx (CPR).

ORIGINAL DESCRIPTION (in Bulgarian). *"Два ♂ и ♀ екземпляри, уловени в гр. Варна по цветовете на божури на 11. V. 1952 г. От Н. Карножицки, и един ♀ екземпляр от гр. Каварна от 15. IV. 1909 г., намерен в сбирката на А. Маркович, притежават следните белези:*

Тялото е черно, при ♀ ♀ широко и масивно. Елитрите бледожълти, до по-тъмни. Първите

5 членчета на антените (с изключение на върха на 5-то членче) са жълти, останалите членчета са черни. Краката са черни, с изключение на пищялите без върха им, които са жълти. Елитрите бледожълти, еднообразно и гъсто пунктирани, покрити с ниско лежащи жълтеникави космици. Всяко крило носи по 6 черни петна: три са разположени успоредно и близо до средния шев на крилото и три странични -близо до страничния ръб на крилото.

Тези до средния шев са: I-то закръглено - пред средата на крилото, II-то неправилно четириъгълно - зад средата на крилото, и III-то кръгло - близо до върха на крилото. От страничните три черни петна двете крайни са по-големи и неправилно удължени, средното е по-малко и закръглено. Средното петно от вътрешната редица и долното крайно от външната редица се разширяват и допират едно до друго. Големина на тялото при ♂ ♂ - 16 мм, при ♀ ♀ - 17 мм.
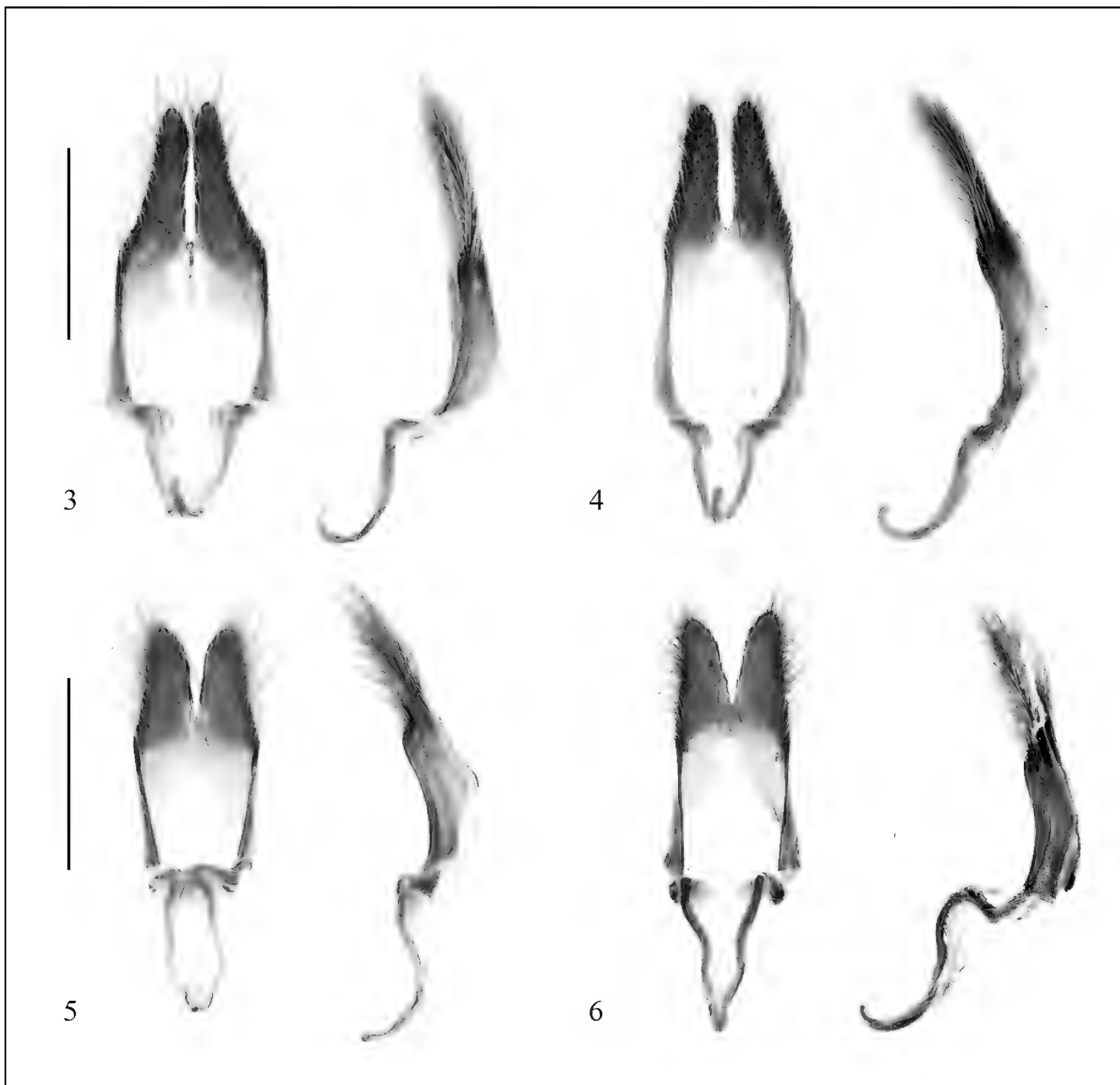
Figures 3–6. Tegmen of *Brachyta bureschi* and *B. balcanica* (ventral and lateral view). Fig. 3: *Brachyta bureschi*: Varna, Karnoschitzky. Fig. 4: *Brachyta bureschi*: Vidno locality. Fig. 5: *Brachyta balcanica*: Burgas, P. Tschorbadjieff. Fig. 6: *Brachyta balcanica*: Asenovgrad locality. Scale bar: 1 mm.

Дължина на елитрите при ♂ ♂ - 11·5 мм.
Дължина на елитрите при ♀ ♀ - 12·8–13 мм.
Находища: гр. Варна - 2 екземпляра (♂ и ♀);
гр. Каварна - 1 екземпляр (♀) - фиг. 1 и 2".

[Translation in English: *Two (♂ and ♀) specimens, found on peony flowers in the town of Varna by N. Karnoschitzky on 11. V. 1952; one ♀ specimen from Kavarna, 15. IV. 1909, from the collection of A. Markovich, with the following characters:*

Body black, in ♀♀ broad and robust. Elytra pale yellow to darker. the first five antennal segments (except for the apex of the 5-th segment) yellow, the rest are black. Legs black, except for tibiae, which are yellow with the exception of the apex. Elytra pale yellow, uniformly and densely punctured, covered with oblique yellowish hairs. Each wing [elytra] with 6 black spots: 3 parallel and close to the wing [elytral] suture and three lateral, close to lateral edge of the wing [elytra].

These [close] to the suture are: the first rounded - before the middle of the wing [elytra], the second irregularly quadrangular - behind the middle of the
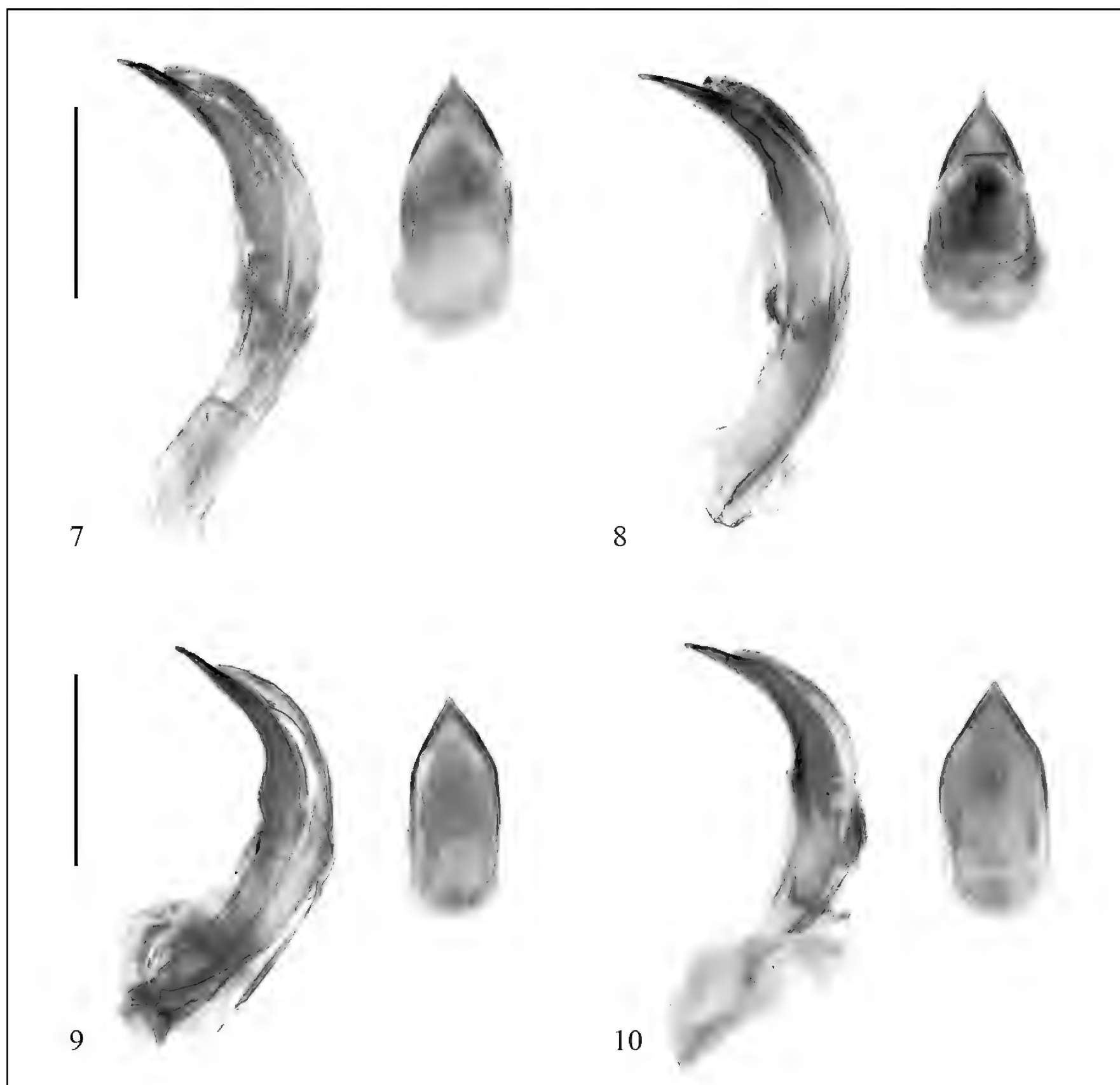
Figures 7–10. Median lobe (penis) of *Brachyta bureschi* and *B. balcanica* (lateral view and apex). Fig. 7: *Brachyta bureschi*: Varna, Karnoschitzky. Fig. 8: *Brachyta bureschi*: Vidno locality. Fig. 9: *Brachyta balcanica*: Burgas, P. Tschorbadjieff. Fig. 9: *Brachyta balcanica*: Asenovgrad locality. Scale bar: 1 mm.

wing [elytra] and the third - close to the apex of the wing [elytra]. Of the three black lateral spots the outer two are larger and irregularly elongated, the middle one is smaller and rounded. The middle spot of the inner line and the lower external spot of the lateral line are expanded and contiguous. Body length in ♂♂ - 16 mm, in ♀♀ - 17 mm.

Elytral length in ♂♂ - 11.5 mm.

Elytral length in ♀♀ - 12.8–13 mm.

Localities: Varna - 2 specimens (♂ and ♀); Kavarna -1 specimen (♀).

VARIABILITY. The black drawing on the elytra is very variable. In all the known species of this group the pattern is very stable and only small variations are known (spots more or less large). In the series of *B. bureschi* that we studied the spots are jointed in different ways, sometimes the elytra being totally black. An interesting variation is that in some specimens all yellow foreground coloration is replaced by a brownish hue where black spots are barely visible. Variations is present in both sexes, but males apparently have a more stable pattern compared with females that are much more variable (Figs. 16, 17).

BIOLOGY. In Romania, adult specimens are known to fly from April to May, or even June (Agigea, 9.VI.1958). We have found specimens only in the second half of April and always on *Paeonia tenuifolia*, in flowers or on the plants where they appear to rest the until second part of the day. We have observed that adults rarely fly and that only in the second part of the day. Adults were observed eating petals or anthers of *Paeonia tenuifolia*. From our observations, copulation takes place also only inside flowers. Rarely, we have seen females on basal part of the plant suspecting that before was on the ground laying eggs. In March 2019, there has been an attempt to search for larva but it had no success. In future, it will be needed to mark the host plant were to try and dig later (end of autumn or very early in spring) for larvae and/or pupae.

In Bulgaria, adults of *Brachyta bureschi* have been collected from the beginning of April to the second half of May. Within the present study, individuals of the species were observed feeding on petals of *Paeonia tenuifolia* L.

The habitat type of both recent species localities in Bulgaria and Romania belongs to Western-Pontic petrophytic steppes (Ponto-Sarmatic steppes). In Bulgaria, the remains of these habitats are only preserved in the northeastern parts of the country and are heavily affected by human activity (Tzonev et al., 2011), which may explain the rarity of this species. In Romania, even if the habitat is known to be a big part of Dobrodja, *Brachyta* is known today only from few locations close to the Bulgarian border. We tried to look for the species also in localities in which it was mentioned historically: Murfatlar, Agigea, Valul lui Traian but without success even if in some of this localities the host plant still exist (Fleck, 1905; Panin & Savulescu, 1961).

Figure 11. Habitat and host plant (*Paeonia tenuifolia* L.) of *Brachyta bureschi* (Bulgaria, Vidno locality).

Figure 12. Habitat and host plant (*Paeonia peregrina* Mill.) of *Brachyta balcanica* (Bulgaria, Asenovgrad locality).

REMARKS. In the historical part of the NMNHS collection eleven specimens of *B. balcanica* are preserved. Of these, only two correspond to the type locality of *B. balcanica* f. *bureschi* (one male and one female).

The male specimen is labeled "STALIN [= Varna] / 24. V. 1953 [?] / KARNOSCHITZKY "/ [backside of the label] Soluk Salaar [Place]// gender symbol.

The locality and the collector (Karnoschitzky = Карножицки) are the same as in the description (Kantardjieva-Minkova, 1957: 542), but the date of collection does not match (11. V. 1952 in the original description). This specimen is not listed either among the types of *bureshi* or in the list of specimens, identified as *Evodinus balcanicus* Hampe (as said abowe, in Bulgarian).

The female fits well with Kantardjieva-Minkova's description. The labels are as follows: 15. IV. [19]09/ Yala Yuch orman [currently Gorichane Vill.]/ Kavarna [in Bulgarian] // *Evodinus balcanicus* Hampe // gender symbol // *Evodinus balcanicus bureschi* - Mink. In the historical collection of the NMNHS, this is the only specimen pinned directly to the label with the name "*Evodinus balcanicus bureschi* - Mink.". The collection date and the main toponym are the same of the third specimen (the second female) in the description paper (in Bulgarian; Kantardjieva-Minkova, 1957: 542). Kantardjieva-Minkova may have not been able to identify the first toponym on the label and therefore did not include it in the description.

For this reason we fixed the female specimen as Lectotype and provided it with a red label with the indication: Lectotype/*Evodinus balcanicus f. bureschi*/Kantardjieva-Minkova 1957/des. P. Rapuzzi, C. Manci and D. Gradinarov, 2019.

According to the ICZN art.45.6.4: *"It is subspecific if first published before 1961 and its author expressly used one of the terms "variety" or "form" (including use of the terms "var.", "forma", "v." and "f."), unless its author also expressly gave it infrasubspecific rank, or the content of the work unambiguously reveals that the name was proposed for an infrasubspecific entity, in which case it is infrasubspecific"*. Since it was described as "*forma*" before 1961 the name should be regarded as available and for this reason it must be accepted as valid.

DISCUSSION

Brachyta bureschi belongs to *Brachyta balcanica* group of species. It differs from *B. balcanica* due to the particular composition of the elytral drawing made by larger black points. The closest species is *B. (Fasciobrachyta) petriccionei* (Figs. 13–15) recently described from Central Italy (Abruzzo region) that can be distinguished by the particular elytral drawing. It is easy to distinguish from the Italian species due to the sparser silver pubescence on the pronotum, denser and golden in *B. petriccionei*. The lateral tooth on pronotum is smaller and shorter and also the disk of pronotum
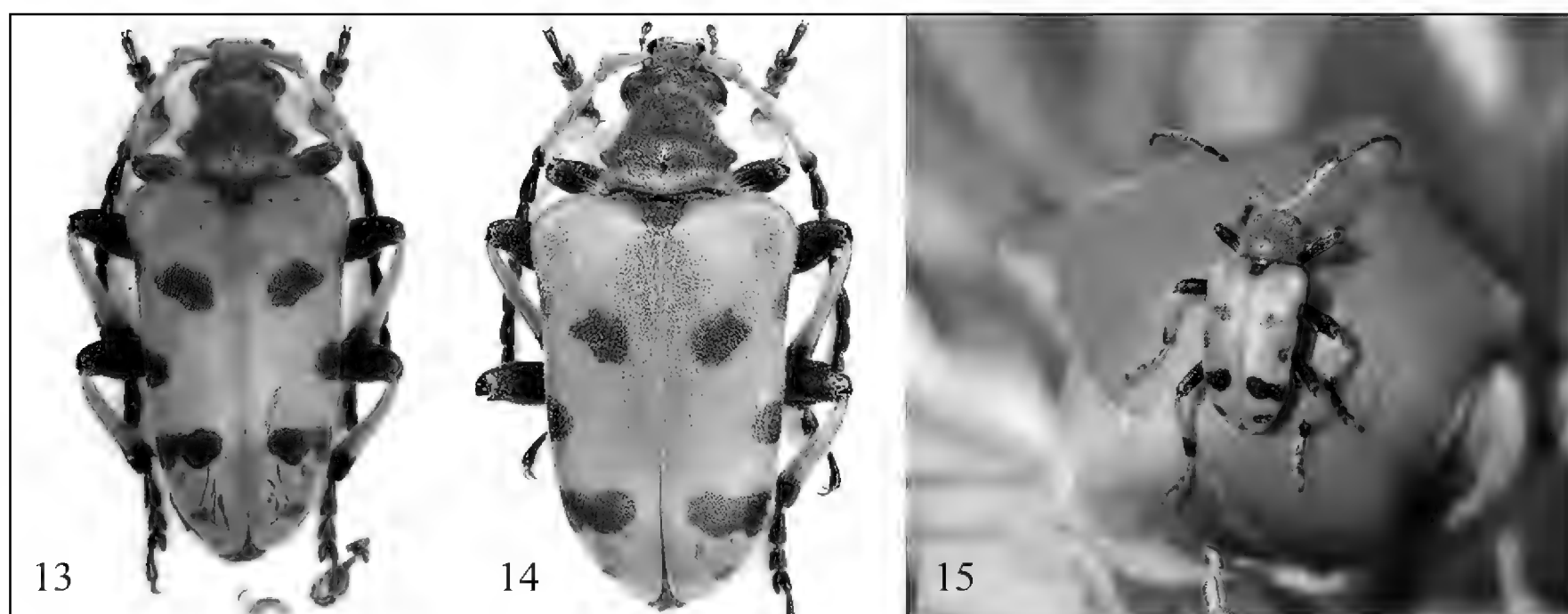
Figure 13. *Brachyta petriccionei* (Italy, Monte Morrone) Holotypus female. Figure 14. Idem, male. Figure 15. Habitat and host plant (*Paeonia officinalis* L.) of *Brachyta petriccionei* (Italy, Monte Morrone) (photo Marco Carafa).
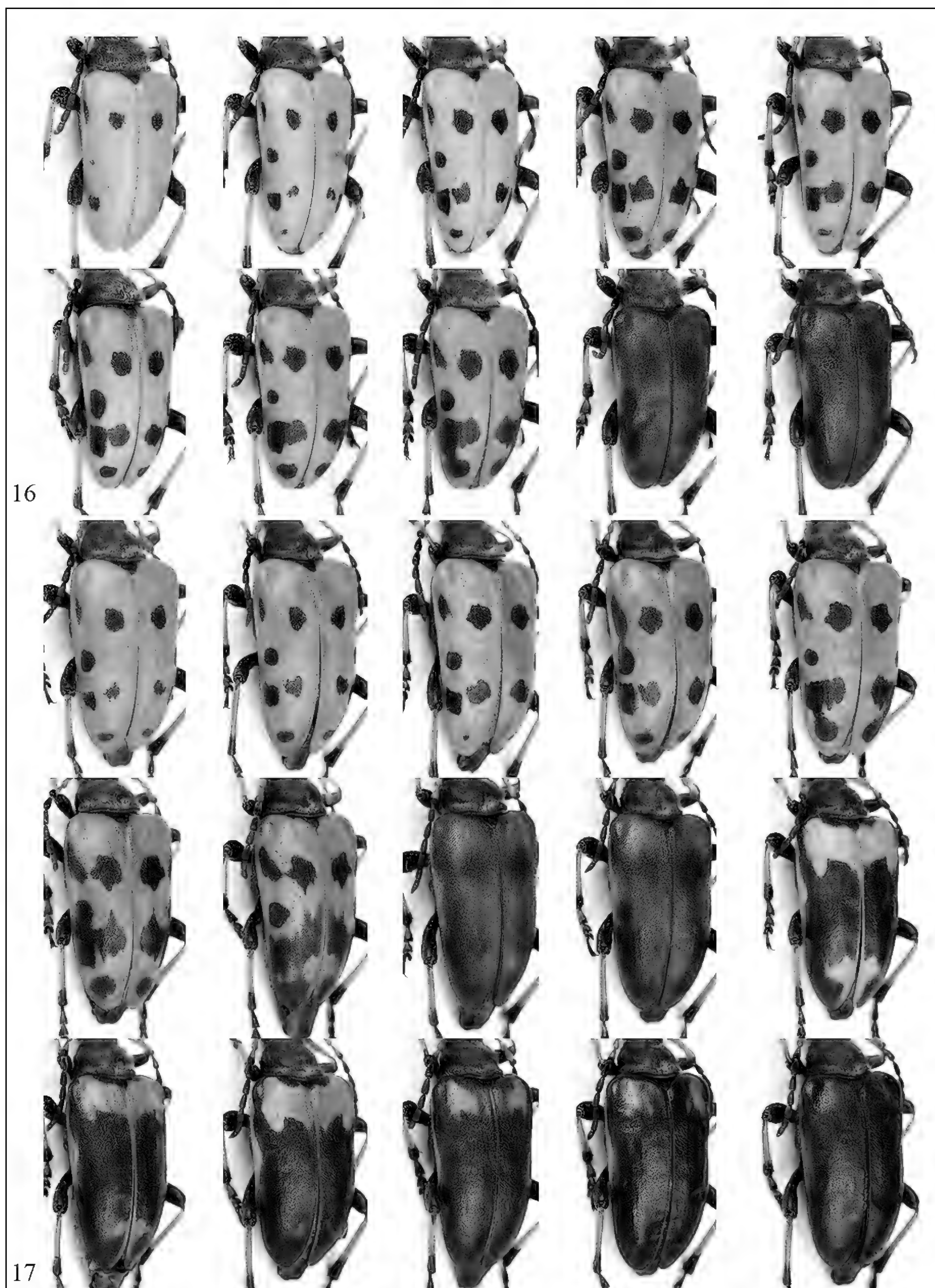
Figure 16. Variability in males of *Brachyta. bureschi* (Romania) (not on scale).
Figure 17. Variability in females of *Brachyta. bureschi* (Romania) (not on scale).
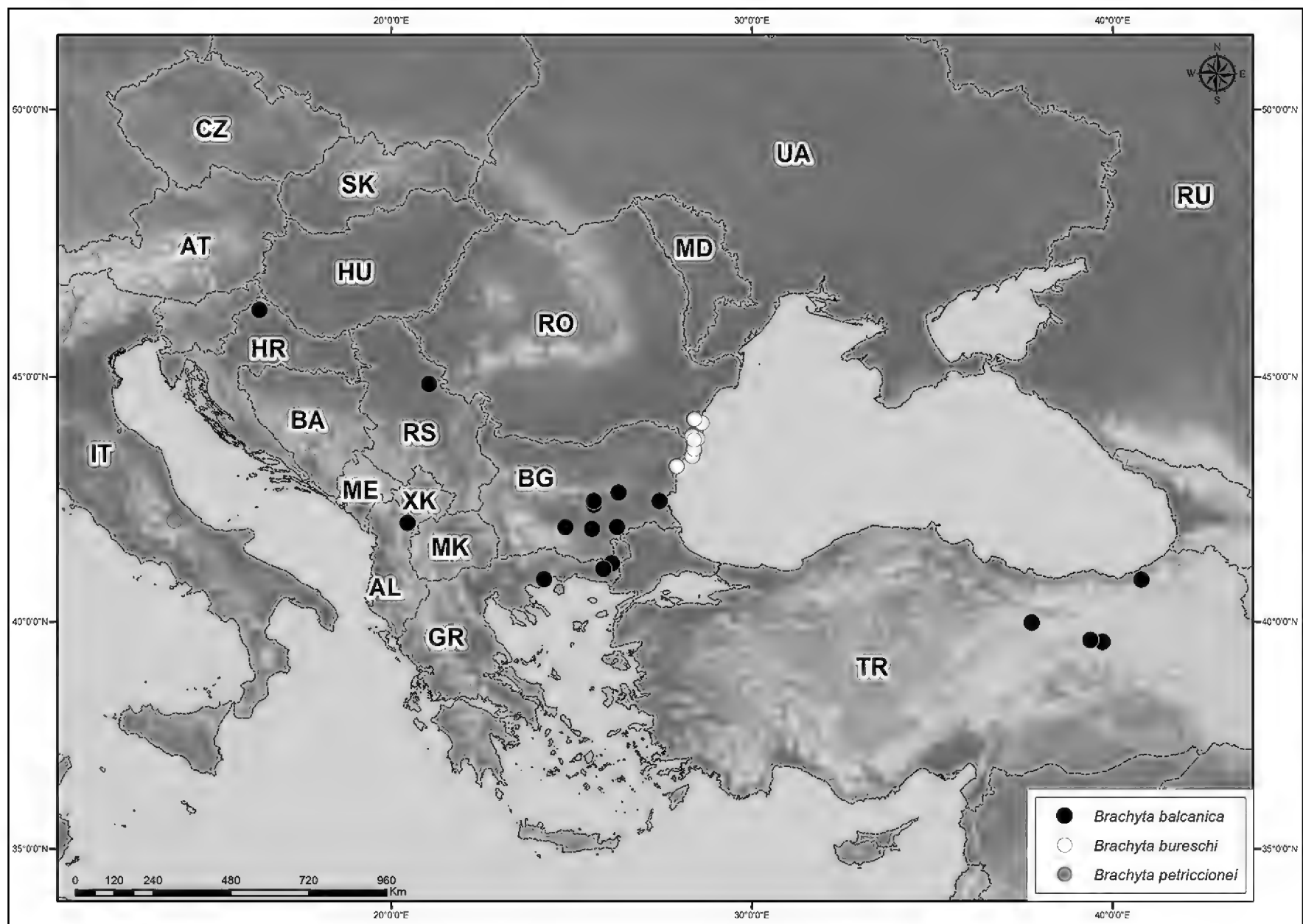
Figure 18. Distribution map of *Brachyta balcanica, B. bureschi* and *B. petriccionei* (based on examined specimens).

is flatter than in *B. petriccionei*. The disk of elytra is flatter and the light ground color is lighter in *B. bureschi*. The two pre-apical points are usually jointed in an incomplete band, interrupted close to the suture; this transversal band is also similar in *B. (Fasciobrachyta) caucasica* (Rost, 1892) but in the Balkan species it is curved towards the base of the elytra instead of horizontal and bent backwards at the outer edge. The ground color is a paler yellow than in all the other species known from this group. The pubescence on the pronotum and on the head in specimens of *B. bureschi* from Bulgaria and Romania, the hairs on the head are clearly lighter than hairs on the pronotum, nearly yellowish is made by short and long thin silvery hairs instead of black as in *B. balcanica*; these hairs are evidently denser and longer than in *B. balcanica*. The elytral punctuation is denser and deeper. The sculpture on the pronotum and on the head is stronger and made by points of different size, more regular in size in *B. balcanica*.

A comparison of the male genitals of several in-

dividuals of *B. balcanica* and *B. bureschi* from Bulgaria was also performed. *B. bureschi* obviously differs from *B. balcanica* by the slender and longer parameres, by the general shape of the tegmen (Figs. 3–6) and by the distinctly pointed apex or the penis (Figs. 7–10). Male genitalia of *B. bureschi* from Romania is similar with the one from Bulgarian specimens.

Apart from the morphological differences, the species appears to have a different host plant. In Bulgaria, *B. bureschi* was observed feeding on *Paeonia tenuifolia* L. (Vidno locality) (Fig. 11), while *B. balcanica* on *P. peregrina* Mill. (Asenovgrad locality) (Fig. 12). *Brachyta petriccionei* was collected on the leaves of *Paeonia officinalis* L. (very likely its host) (Fig. 15). In Romania, from our knowledge only *B. bureschi* exist and even in a locality, Coroana, where both species of *Paeonia* (*tenuifolia* and *peregrina*) co-occur, the species was found to be only on *Paeonia tenuifolia* and no specimens being found on *Paeonia peregrina*.

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First record of the Northern white-breasted hedgehog *Erinaceus roumanicus* Barrett-Hamilton, 1900 (Mammalia Erinaceidae) in the Aegean island of Chalki (Dodecanese, Greece)

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ABSTRACT   The presence of the Northern white-breasted hedgehog *Erinaceus roumanicus* Barrett-Hamilton, 1900 (Mammalia Erinaceidae) for the Aegean island of Chalki (Dodecanese, Greece), is here recorded for the first time. *E. roumanicus* is common in many Aegean islands, however, his presence in Chalki had never been reported.

KEY WORDS   Northern white-breasted hedgehog; Chalki; Dodecanese; *Erinaceus roumanicus*; Rhodes Archipelago.

INTRODUCTION

Erinaceus roumanicus Barrett-Hamilton, 1900 (Mammalia Erinaceidae) has a global distribution extending from central and eastern Europe, the Baltic and the Balkan Peninsula eastwards through Belarus, Ukraine, and Russia, reaching as far as western Siberia. In the south, its range extends as far as the northern Caucasus and the island of Crete. It is recorded from sea level to at least 1,400 meters (Lapini, 1999). Within the Mediterranean region, it ranges from Italy and Slovenia, through the Balkan Peninsula (Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Albania, Macedonia, Bulgaria, Greece, and European Turkey) and extending south into the Near East Anatolian Turkey. The northern white-breasted hedgehog inhabits farmland, parks and gardens in rural and urban areas, scrubby habitats at the edge of forests, and shrubby vegetation.

Like its congener *E. europaeus*, it is more abundant in artificial than in natural habitats (Lapini, 1999). Many are killed by collision with cars (Lapini, 1999), but this is unlikely to cause widespread population declines (Amori et al., 2010). Hedgehogs consume a variety of food, but mainly of animal origin: different invertebrates, small vertebrates (rodents, amphibians, reptiles, eggs of birds and their young). Of secondary importance are fruits, mushrooms and other (Peshev et al., 2004).

According to a morphological study carried out by Kryštufek et al. (2009), insular hedgehogs tend to be smaller on remote islands and larger on islands close to the mainland coast, which is their putative source of colonization. However, the insular response is not uniform and the large size close to the mainland is possibly a result of frequent introduction (Masseti, 2012).

Several subspecies have been described from the European range of the northern white-breasted hedgehog; six subspecies were reported from continental and insular Greece. Chalki specimens should belong to the subspecies *E. roumanicus rhodius* Festa, 1914 that is present in the neighboring island of Rhodes.

MATERIAL AND METHODS

Study area

Chalki is a small island belonging to the Dodecanese Archipelago (south-eastern Aegean Sea), located south-west of Rhodes Island. Its coordinates are 36°13'44.49" N; 27°34'18.74" E. Administratively Chalki is part of Rhodes Regional Unit. Its located west of Rhodes (Monolithos Cape), from which is only five nautical miles, southeast of Tilos (10 nautical miles), and north of Karpathos. It has a length of 10 km, a width of 4 km and an area of 28,125 km². The wider and more important bays are Imborios and Pondamos (Iliadis, 1950). Chalki is a mountainous and rocky island and Maistros Mount (593 m a.s.l) represents the highest peak. The entire island, except for the eastern part, is surrounded by very steep cliffs with scarce accessibility. These vertical cliffs allowed the presence of a rare and highly specialized chasmophytic flora. Several species are endemic with a distribution area limited to the island and to the south-eastern Aegean, including western Turkey (Cattaneo & Grano, 2014; Cattaneo & Grano, 2015). Recently, a new species for science that seems to be restricted to Chalki and the nearby island of Tilos has been discovered: *Seseli halkensis* Cattaneo, Tan et Biel (Cattaneo et al., 2016). The island is essentially dry, and lacks of superficial hydrography, however, the numerous wells on the island are still rich in water. In ancient times Chorio (the current Palio Chorio) was the capital, village by now abandoned. Currently the population, which amounts about three hundred inhabitants, is concentrated exclusively in Imborios, which appears to be the only village on the island. Together with the small surrounding islands, Chalki is included in the Natura 2000 network (GR 4210026) for the presence of particular bird species and for a peculiar chasmophytic flora related to elective habitats represented by crevices in the limestone rocks of these islands (Cattaneo & Grano, 2014; Cattaneo & Grano, 2015).

Methods

The author and his wife Cristina Cattaneo investigated the island of Chalki in four different times, during August 2014, April 2015, August 2016 and August 2017. The first investigations were also extended to the neighboring island of Alimia. Researches have brought important discoveries to this island for flora (Cattaneo & Grano, 2014, 2015, 2018; Cattaneo et al., 2016), theriological fauna (Grano et al., 2015), reptiles (Grano & Cattaneo, 2015, 2017; Grano et al., 2015) and land snail (Liberto et al., 2017). Despite these researches, the presence of the hedgehog on the island has never been ascertained. Only recently have specimens been sighted on the island. Some alive and only one hit by a car. The example of figure 1 was photographed in a road off the main road to Pondamos beach, near the school by Daniel Foulkes-Jones.

RESULTS AND CONCLUSIONS

Masseti & Sara (2003) and Masseti (2012) consider that this species has been introduced in all the Aegean islands where they are found, imported for supply of food and to control snakes. In fact, this small mammal is present in most of the Greek Aegean islands. The reasons why it has never been reported before is due to several reasons: Chalki is a small island with few inhabitants all residing in the same village, the island is particularly arid and cultivated fields and gardens are practically inexistent, except for a few very small personal cultivation near the houses. Only one road is accessible for the few cars on the island and therefore kill-road finds are very rare. One of the reasons some hedgehogs have been seen during this period may be due to the tranquility that all wildlife has had due to the lockdown of activities and tourism due to the COVID-19 pandemic.

Indeed, while a drop in transportation during the coronavirus lockdowns led to lower pollution levels around the world, the slowdown in tourism also lowered another big problem: the massive presence of humans in natural areas.

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Figure 1. *Erinaceus roumanicus* from Chalki, Dodecanese Archipelago (south-eastern Aegean Sea).

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Introduction of microscopic non-indigenous species through ballast water in Arzew Gulf (SW Mediterranean Sea): the case of the harmful raphidophyceae *Fibrocapsa japonica* S. Toriumi et H. Takano, 1973 (Chattonellaceae)

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ABSTRACT   The transfer of non-indigenous species (NIS) into the marine environment is mainly carried out by maritime transport (biofouling or ballast water). Ports are therefore the gateway to NIS, which can alter local biodiversity. In this study, the port of Arzew and Bethioua (Algeria, south-western Mediterranean) were studied for the presence of NIS by taking the case of the harmful Raphidophyceae *Fibrocapsa japonica*. Seawater samples were collected following a comparative sampling strategy between Arzew Gulf (AG region: presence of two hydrocarbon transport ports) and Ain Temouchent (AT region: absence of transport port - reference area). The Almeria-Oran front separates the two regions. The AT and AG regions generally have the same temperature, salinity and chlorophyll characteristics. The phtyhoplankton population showed a homogeneous quantitative distribution between the AT and AG regions. Diatoms and dinoflagellates have an equal density in both regions, nevertheless diatoms are more abundant in each region, (diatoms 6950 ind/l in AT 6380 ind/l in AG, dinoflagellates 1920 ind/l in AT and 1770 ind/l in AG). The specific assessment of the phytoplankton population revealed the presence of *F. japonica* at around 110 ind/l in the AG region in one station across the ports of Arzew and Bethioua and near the coast, and its absence in the reference area AT. Despite its presence in low density in a single station, this species remains harmful and presents a real danger if it is transported by the Algerian current along the coast to other places, in particular fisheries and aquaculture production areas. This study is part of a prevention process, is the first signaling of microscopic non-indigenous species on the Algerian coast, and contributes to establish a database for future assessments of microscopic NIS in Algerian ports.

KEY WORDS   NIS; Arzew Gulf; ballast water; *Fibrocapsa japonica*; harmful algae.

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INTRODUCTION

Since it was first reported in USA and Japan, the Raphidophyceae *Fibrocapsa japonica* (S. Toriumi et

H. Takano, 1973) has attracted the attention of several researchers and has been studied for its ecology and toxicity, which can cause fish kills (Iwasaki, 1971; Toriumi & Takano, 1973; Okaichi,

1989; Hiromi et al., 1995; Cortés-Lara et al., 2003; Vershinin & Orlova, 2008; Härnström et al., 2009; Engesmo et al., 2018). Several mechanisms are responsible for this toxicity: production of brevetoxins, fatty acids and reactive oxygen species (ROS) (Khan et al., 1996a, b), an abundant production of mucous that clogs fish gills and the production of haemolytic compounds (Fu et al., 2004). At present, *F. japonica* has a wide geographical distribution (Fig. 1, Table 1) and is a typical temperate region species that cannot survive in Polar Regions (de Boer et al., 2005). Kooistra et al. (2001) suggest that anthropogenic activity (ship ballast water exchange and/or aquaculture) may have induced the expansion of the disjunct range of *F. japonica*. In addition, de Boer et al. (2005) concluded that the presence of *F. japonica* in the North Sea is of anthropogenic origin linked to ballast water exchange, as the physiology of strains observed in the North Sea is similar to the physiology of strains observed in the northern Pacific Ocean, although there is no direct contact between these waters.

In Alboran Sea (West Mediterranean basin), this species was detected for the first time in autumn 2006 by Fani et al. (2009). This basin covers the Algerian west coast where a study was carried out in our laboratory by Bouda et al. (2015) who estimated the risk of macroscopic species introduction by ballast water in the port of Arzew, with 76 donor ports of which 29 represent a high risk, 34 a medium risk and 13 a low risk. Previous work by researchers on *F. japonica* and the study of the risk of ballast water in Arzew gulf have led us to ask the following questions: can we validate the risk of introducing exotic species into Arzew gulf on a microscopic scale? Can we link the presence of *F. japonica* to ballast water?

The aim of our work is to respond to this problem in order to provide new data that could explain the processes of introduction of microscopic species in Algeria. Our study thus constitutes the first recording work of microscopic species introduced into Algerian waters, and could be a starting point for the realization of an inventory of introduced microscopic marine species in particular harmful phytoplankton. This study and the results of future related research will contribute to the enrichment of the first assessment of macroscopic species introduced into Algerian waters between 1834 and 2017 established by Grimes et al. (2018) and the first records of non-indigenous species in port of Arzew indicated by Bensari et al. (2020). All of this within a common prevention framework and to take "*appropriate measures to regulate the in-*
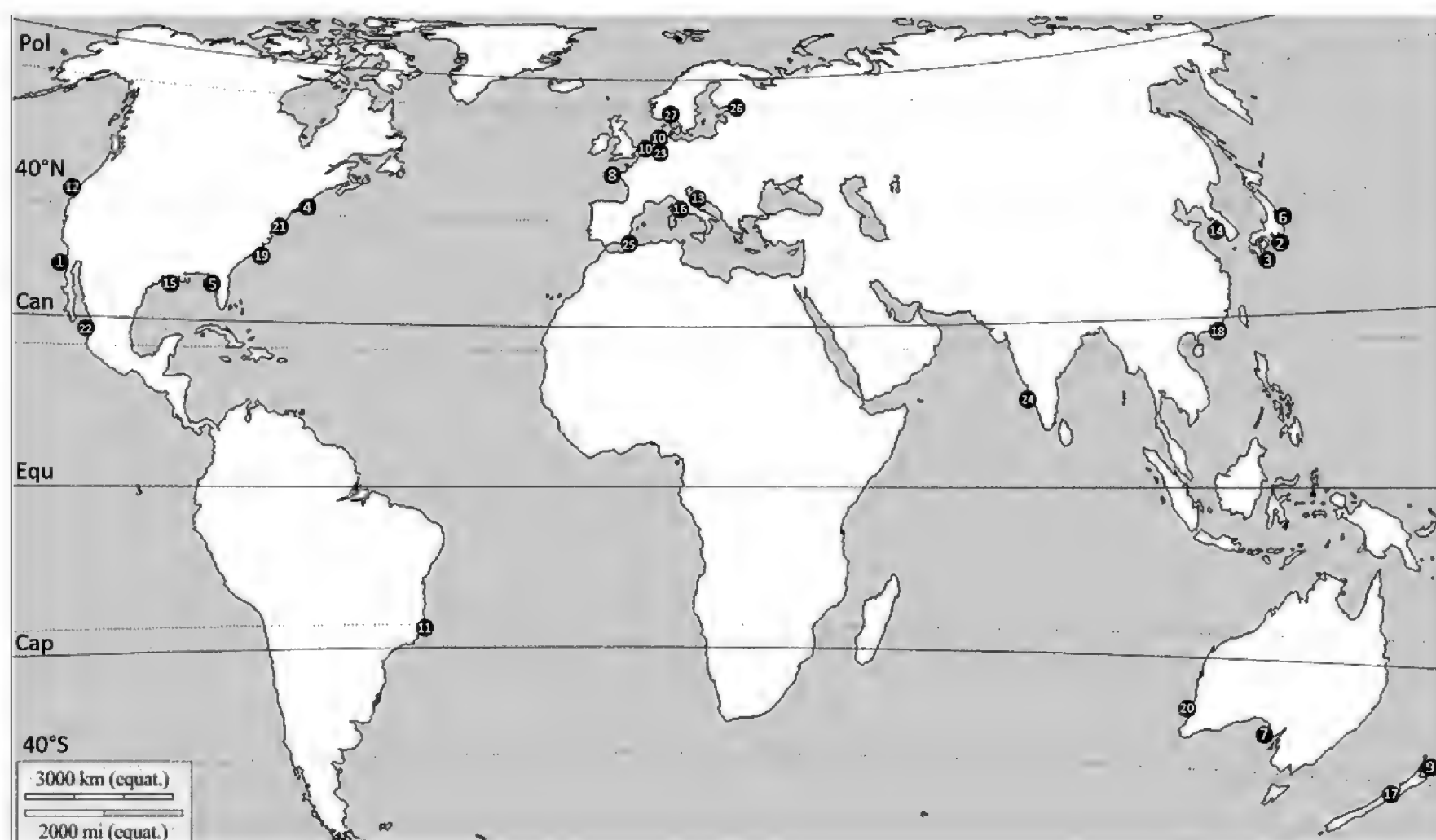

Figure 1. Distribution of *Fibrocapsa japonica* (modified from de Boer et al., 2004).

tentional or accidental introduction of non-indigenous or genetically modified species into the wild and prohibit those that may have harmful impacts on the ecosystems, habitats or species", as required by the Barcelona Convention through its Protocol concerning specially protected areas and biological diversity (UNEP-MAP RAC/SPA, 2005), which were supported by Decision IG.22/12 related to "Species Introductions and Invasive Species" (UNEP/DEPI) /MED WG.421/26) (Grimes et al., 2018).

MATERIAL AND METHODS

Study area

In order to respond to the problems posed previously, our study is made in the geographical framework of Fani et al. (2014) and Bouda et al. (2015) work, namely the Algerian west coast. The ability to detect non-indigenous species (NIS), especially those of low abundance, is limited by the lack of data and information on phythoplankton species on the Algerian coast. Therefore we opted for a traditional method of detecting non-native species based on the comparison of two distinct regions of the western region (Fig. 2).

A distance of about 80 km separates the two areas, which corresponds to the geographical location of the Almeria-Oran front (Fig. 2). This front constitutes an ecological barrier that prevents the mixing of water bodies between the two zones. Several researchers in their recent studies in the region have used this sampling strategy (e.g., Diz & Presa, 2008; García-Merchán et al., 2012; Riesgo et al., 2016; Pascual et al., 2017).

Arzew Gulf (AG): mainly chemical industries and large petrochemical slabs are present along the coast in the industrial poles of Arzew and Bethioua which are the main gateway for hydrocarbons exported from Algeria (Fig. 3). Most of ships berthing discharge systematically their ballast water. That is why the Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea "REMPEC" report of 2008 classifies Arzew port among the most vulnerable ports, in the Mediterranean Sea, to such type of risk (Bouda et al., 2015).

Ain Temouchent (AT): this zone is essentially agricultural, bordered by tourist beaches and a single fishing port. There is no industrial activity and no ballast water discharge. For this reason this zone is considered a reference in our study.

Sampling

The study was based according to basic data collected during the prospecting campaign of the Algerian demersal resources "ALDEM 2017" conducted on July 2017 in partnership with the CNRDPA (National Center for Research and Development Fisheries and Aquaculture). Fourteen seawater samples were collected: 6 stations are located in Ain Temouchent and 8 stations are located in the Arzew Gulf (Fig. 3).

Phytoplankton sampling and analysis

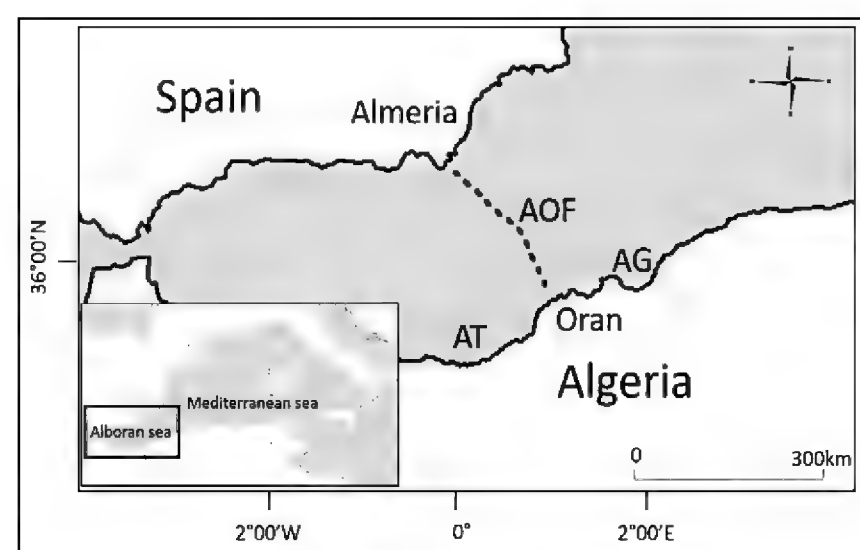Seawater samples were collected from surface

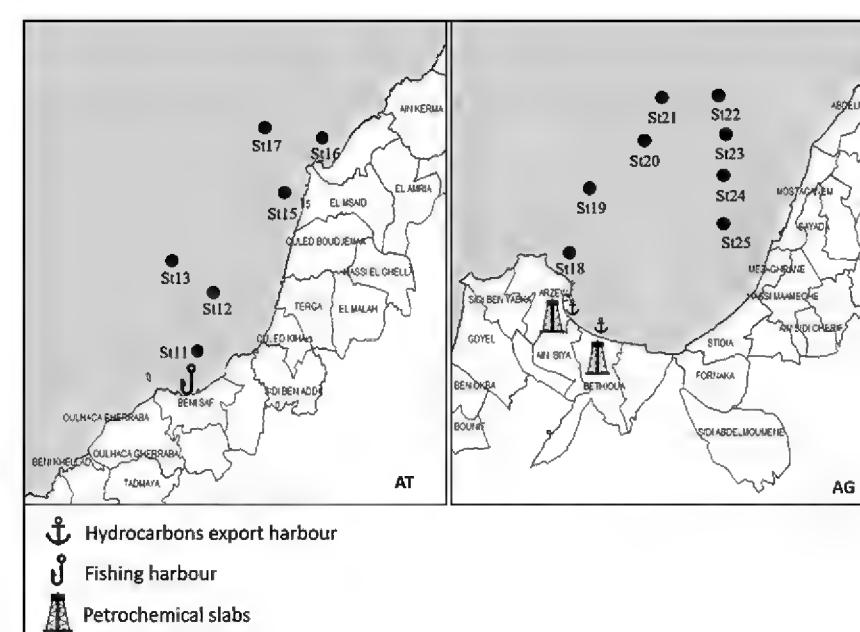Figure 2. Sampling area in AG (Arzew Gulf); AT (Ain Temouchent). AOF: Almeria Oran Front.

Figure 3. Sampling stations position
AG (Arzew Gulf) and AT (Ain Temouchent).

water using a 5L Niskin bottle mounted on a graduated rope. The sampled water was then transferred to a clean, dark polypropylene (PP) bottle with a volume of 1L. Phytoplankton samples are immediately fixed with an alkaline solution of Lugol with about 5 ml per 1L, and are stored in a dark and cool place (4 °C).

The technique used for sample preparation and phytoplankton identification and enumeration is that of Utermöhl (1958). This method consists of reading the sample under an inverted microscope (objective 40x, OPTIKA) with camera by counting the phytoplankton cells. The identification was carried out at the specific level or, in case of difficulty or uncertainty, at higher taxonomic level (genus, family or class) using different identification keys (Graham, 1942; Trégouboff, 1957; Taylor, 1976; Balech, 1988). The results were expressed in number of individuals per unit volume (ind/l).

Temperature and salinity measurement

These factors are the mean of the measurements taken each meter between 1 m and 20 m from the surface, using the Conductivity-Temperature-Depth (CTD) instrument set. Salinity is given by the PSU (Practical Salinity Unit) and temperature by degrees Celsius (°C).

Chlorophyll

The MODIS AQUA (EOS PM-1) sensor is chosen to create the data base for this spectral resolution with 9 bands which are used to observe the color oceans.

Satellite images are downloaded every 5 km around each station to extract Chl values. The download was done on July 2017 by using the NASA Ocean Color website (http://reason.gsfc. nasa.gov/Giovan-ni) to access to database of GSFC (Goddard Space Flight Center) of NASA (National Aeronautics and Space Administration).

Images downloaded were then selected in clear sky and then treated on three stages:

- Calibration: it consist to transform reflectance values to numerical values by using algorithms integrated in ENVI software: OC3 algorithm for extracting chlorophyll concentrations.

- Geo-referencing: to have the punctual geographic coordinates according to the chosen stations by using projection system Geodesie WGS-84.

- Extraction of the information: by using the software ENVI 4.8, data were displayed by clicking on pixel under a clear sky. Displayed values were used to calculate the averages (mg/l).

Numerical analysis

Student test. The question is whether the means of the two groups are statistically significantly different when comparing the means. The calculation was done on Excel stat.

Shannon index. The Shannon diversity index (H) is commonly used to characterize species diversity in a community. Shannon's index accounts for both abundance and evenness of the species present. It is given by:

$$H = -\sum_{i=1}^{s} p_i \ln p_i \text{ with } Pi = \frac{n_i}{N}$$

Chi-square test. This tests the hypothesis of independence of two categorical variables. If they

N°	Date	Place	Reference
22	2003	Matanchen Bay, Nayarit, Mexican Pacific coast	Cortes *et al.* (2003)
23	2004	Antwerp port, Belgium	Clark *et al.* (2004)
24	Jan-06	Karnataka old port, Arabian sea, India	Härnström *et al.* (2008)
25	Autumn 2006	Eastern Alboran Sea, Mediterranean sea	Fani *et al.* (2009)
	Jul 2017	Western Algeria	Ali *et al.* (2020)
26	2007	Baltic sea, Russia	Vershinin & Orlova (2008)
27	2009–2012	Oslofjorden, Norway	Engesmo *et al.* (2018)

Table 1. First sightings of *Fibrocapsa japonica* in the worlds (the numbers correspond to figure 1). Modified from De Boer et al., 2004.

share something in common, one variable influences the other.

$$X_c^2 = \sum_{i=1}^{K} \frac{(oi-ei)^2}{ei}$$

c = Degrees of freedom; o_i = Observed value (s); e_i = Expected value (s).

RESULTS

The figure below represents the July 2017 mean Chl-a (mg/l) and measurement in-situ of temperature (°C) and salinity (PSU) in stations for each region. The Chl-a concentration in AT varies between 0.15 mg/l and 0.19 mg/l. Although the difference between the maximum and minimum values is minimal (0.04 difference), the positioning of the values at each station shows a decreasing gradient from coast to offshore. In the other hand, the chl-a concentration in the Arzew Gulf varies between 0.15 mg/l and 0.25 mg/l.

The values positioned at each station show a particular pattern with increasing values from station 25 to station 18, noting stability at stations 22, 23 and 24 (Fig. 4). In addition, we note that the radial with stations 18 to 21 is richer in Chl-a. The temperature varies between 22.7 °C and 24.3 °C at AT with an increasing temperature gradient from wide to offshore.

In the AG region, the temperature varies between 21.8 °C and 23.5 °C (this is the maximum at station 22). There is an increasing gradient on the radial 18–21 and a decreasing gradient on the radial 22–25. In general, AT is warmer than AG. Overall salinity remains stable at about 36.5 PSU at all stations in each region. We note the highest value (36.8 PSU) at station 22.

In Table 2, quantitative phytoplankton groups distribution per region can be seen.

AT: In all stations, diatoms are more abundant than dinoflagellates, except station 13 where dinoflagellates are clearly more dominant. Comparing this station with the nearest station (St 12), separated by a distance of 8.7 km, an inversion in the distribution of the two groups is observed. Indeed, in station 12 diatoms are very abundant with 3630 ind/l against a low presence of dinoflagellates estimated at 120 ind/l. Conversely, in station 13 dinoflagellates are more abundant with a contribution of 910 ind/l against 170 ind/l for diatoms.

	St11	St12	St13	St15	St16	St17
S	36.5	36.6	36.6	36.6	36.5	36.5
T	23,6	23.6	23.9	22.7	23.3	24.3
Chl	0.19	0.17	0.15	0,19	0.18	0.16

	St18	St19	St20	St21	St22	St23	St24	St25
S	36.5	36.51	36.5	36.5	36,8	36.6	36.5	36.5
T	23.3	23.03	22,8	22.6	23,5	22.5	22.3	21,8
Chl	0.25	0.22	0,19	0.18	0.17	0.17	0.17	0,15
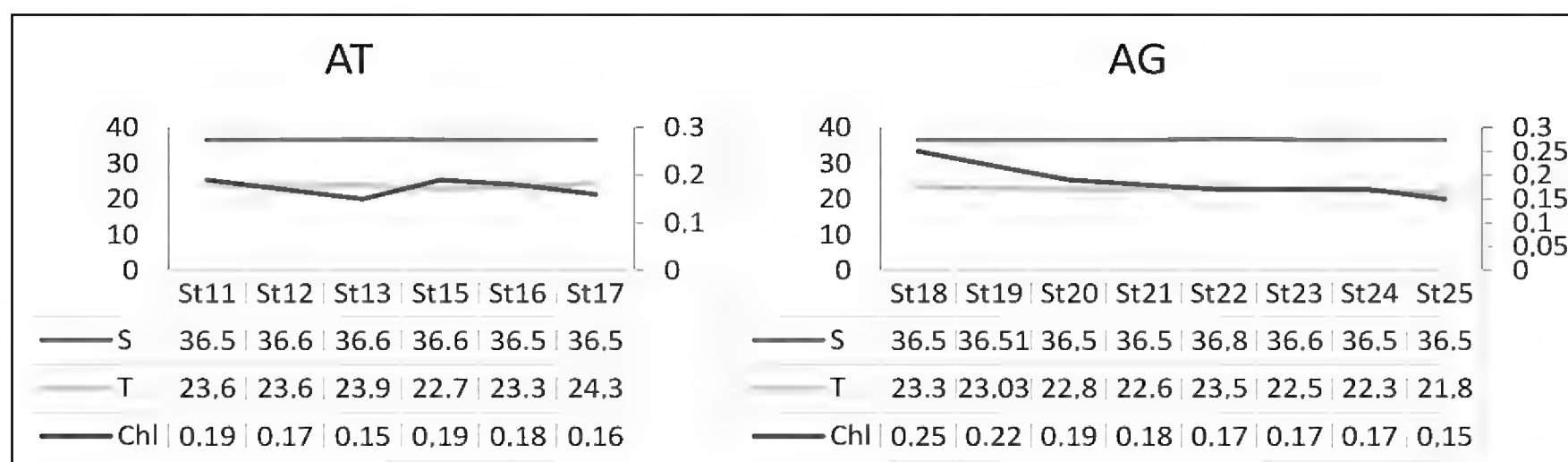
Figure 4. Chl-a (mg/l), temperature (°C) and salinity (PSU) values in stations for each region.

	AT							AG						
Stations	St11	St12	St13	St15	St16	St17	St18	St19	St20	St21	St22	St23	St24	St25
Dinoflagellates	110	120	910	120	310	350	160	260	270	70	290	10	200	510
Diatoms	550	3630	170	1020	620	960	1130	150	170	120	2870	70	680	1190

Table 2. Phytoplankton groups distribution in AT and AG stations (ind/l).

AG: Diatoms are more abundant in most of the stations except St19 and St20 where dinoflagellates are more abundant. This distribution follows the shape of the bay, forming an arc for each phytoplankton group (diatoms near the coast and dinoflagellates offshore).

The Student test shows that AT is richer in both dinoflagellates and diatoms then AG. However, this richness remains insignificant since the calculated p-value is higher than the significance level threshold alpha = 0.05, thus we cannot reject the null hypothesis H_0 (Fig. 5).

Shannon Index application indicates that AG is slightly more diverse in dinoflagellates than the AT region, with Ish = 3.6 ind/bit and Ish = 3.1 ind/bit respectively. Nevertheless, the regularity index shows that this group is ecologically equitable in both regions with a homogeneous distribution and no specific dominance (0.8 < R > 0.9). Conversely, AT has a higher diatom diversity compared to AG, with respectively Ish = 2.4 ind/bit and Ish = 1.7 ind/bit. The regularity index shows that AT is fair with a homogeneous distribution of diatoms (R = 0.7), while AG presents a moderately homogeneous distribution with R = 0.5.

The diversity index calculated per station of each group shows that station 13 is the most diverse with Ish = 2.97 ind/bit, while station 22 is the least diverse with Ish = 1.02 ind/bit. The lowest regularity index (R = 0.3) is noted in this station, indicating the presence of specific dominance. Indeed, the genus *Chaetoceros* contributes to more than 90% of dinoflagellates, i.e., 2600ind/l. On the other hand, the highest R index is noted in station 23 (R =1) fol-

lowed by station 21 and 20, with R = 0.96 and R = 0.71 respectively.

On the other hand, extreme values are given by certain genera of each group in each zone, which raises the question of whether or not the abundance of the groups is region-dependent, i.e., whether the region influences the abundance of phytoplankton groups. To answer this question the Chi-square test was applied.

Chi-square Test application. H_0: The distribution of phytoplankton groups does not depend on the region.

H_1: The distribution of phytoplankton groups depends on the region.

X^2 Calculated = 0.013.

X^2 Table = 3.84 for a d.d.l = 1 and α = 5%

X^2 Cal << X^2 tab.

H_0 retained: the distribution of phytoplankton groups does not depend on the region, showing that they are homogeneously distributed in both regions.

The presence of dinoflagellates is 38% and 41% for AT and AG respectively, these values which are relatively close indicate that this group has a homogeneous distribution in these two sites. This is also observed for diatoms, with 10% in AT against 11% in AG.

The overall quantitative analysis of the main phytoplankton groups shows that they are similarly distributed in the regions studied. This indicates that the characteristics of each area do not influence the abundance of dinoflagellates and diatoms.

A specific qualitative analysis of diatoms and dinoflagellates was necessary to determine the characteristic species in each area.

For the qualitative phytoplankton groups distribution per region, Table 3 shows that the AT and AG regions each have 13 genera of diatoms (without taking into account unidentified individuals), with the absence of the genera *Cosinodiscus*, *Pinnularia* and *Pleurosigma* in AG, and the absence of *Detonula*, *Fragilaria* and *Thalassionema* in AT. However, *Chaetoceros* are dominant in AG with 4170 ind/l compared to 1380 ind/l in AT. Furthermore, the genera *Cylindrotheca*, *Dactyliosolen* and *Leptocylindrus* are dominant in AT with 870 ind/l, 1020 ind/l and 2370 ind/l respectively compared to 250 ind/l, 480 ind/l and 560 ind/l in AG. On the specific scale, AG has 26 species compared to 32 species in AT.

We report the presence of some harmful diatoms: *Cylindrotheca closterium* (produce mucilage:
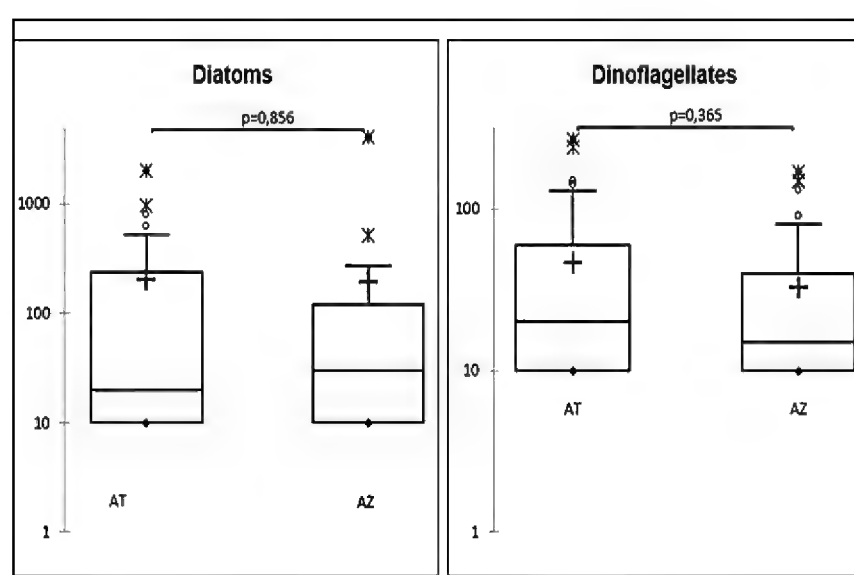

Figure 5. Box plot for diatoms and dinoflagellates. H_0: The difference between the averages = 0. H_1: The difference between the averages $\neq$ 0.

Genus	Species	AT	AG	Genus	Species	AT	AG
Chaetoceros	*ceratosporus*	+	-	*Nitzschia*	*seriata*	+	-
Chaetoceros	*convolutus*	+	+	*Nitzschia*	*sp*	-	+
Chaetoceros	*danicus*	+	+	*Pinnularia*	*sp1*	+	-
Chaetoceros	*compressum*	+	+	*Pinnularia*	*sp2*	+	-
Chaetoceros	*didymus*	+	+	*Pinnularia*	*sp3*	+	-
Chaetoceros	*sp2*	+	-	*Pinnularia*	*sp4*	+	-
Chaetoceros	*sp3*	-	+	*Gyrosigma*	*sp*	+	-
Coscinodiscus	*sp*	+	-	*Pseudo-nitzschia*	*seriata*	+	+
Cylindrotheca	*closterium*	+	+	*Pseudo-nitzschia*	*delicatissima*	-	+
Dactyliosolen	*fragilimus*	+	+	*Rhizosolenia*	*sp1*	+	+
Dactyliosolen	*sp1*	+	-	*Rhizosolenia*	*styliformis*	+	+
Dactyliosolen	*sp2*	-	+	*Rhizosolenia*	*sp3*	+	+
Dactyliosolen	*sp3*	-	+	*Rhizosolenia*	*setigera*	-	+
Detonula	*sp*	-	+	*Thalassionema*	*nitzschioides*	-	+
Achnanthes	*coarctata*	-	+	Unidentified	*sp1*	+	-
Guinaridia	*striata*	-	+	Unidentified	*sp2*	+	-
Leptocylindrus	*minimus*	+	+	Unidentified	*sp3*	+	-
Leptocylindrus	*danicus*	+	+	Unidentified	*sp4*	+	-
Leptocylindrus	*sp*	+	-	Unidentified	*sp5*	-	+
Licmophora	*abbreviata*	+	+	Unidentified	*sp6*	-	+
Navicula	*sp*	+	+	Unidentified	*sp7*	-	+
Nitzschia	*amphibia*	+	+	Unidentified	*sp8*	-	+
Nitzschia	*closterium*	+	-	Unidentified	*sp9*	-	+
Nitzschia	*longissima*	+	+	Unidentified	*sp10*	-	+
Unidentified : isolated cells < 20 ind/l							

Table 3. Diatoms population in AT and AG regions. Unidentified: isolated cells < 20 ind/l.

Kraberg et al., 2010), *Dactyliosolen fragilimus* (clog fills of benthic shelfish: Lorrain et al., 2000) *Guinaridia striata* (impact copepod reduction: Wichard et al., 2008), *Pseudo-nitzschia seriata* and *P. delicatissima* (amnesic shellfish poison: Hasle & Syvertsen, 1997), and *Rhizosolenia setigera* (anoxic conditions, mortalities of marine organisms).

We notice that the sum of these harmful diatoms in the AT region exceeds that of the AG region, i.e., 2750 ind/l against 1090 ind/l, respectively.

We note the presence of 21 genera of dinoflagellates distributed into 20 genera in AG and 14 genera in AT (without taking into account unidentified individuals), with the absence of the genera *Alexandrium*, *Fibrocapsa*, *Gonyaulax*, *Gonyostomum*, *Ornithocercus*, *Oxytoxum* and *Pyrodinium* in AT. Only the genus *Scripsiella* is absent in AG (Table 4).

There is no dominance of genus or species of dinoflagellates in either region. The highest value is

Genus	Species	AT	AG	Genus	Species	AT	AG
Alexandrium	*sp*	-	+	*Protoperidinium*	*sp1*	+	-
Amphidoma	*caudata*	+	+	*Protoperidinium*	*sp2*	+	-
Amphidoma	*languida*	+	+	*Protoperidinium*	*sp3*	+	-
Azadinium	*sinosum*	+	+	*Protoperidinium*	*sp4*	-	+
Ceratium	*arcuatum*	+	+	*Protoperidinium*	*steinii*	+	+
Ceratium	*candelabrum*	+	-	*Protoperidinium*	*diabolus*	+	+
Ceratium	*extensum*	+	-	*Protoperidinium*	*tenuissium*	-	+
Ceratium	*furca*	+	+	*Protoperidinium*	*sournai*	-	+
Ceratium	*fusus*	+	+	*Protoperidinium*	*sp1*	+	-
Ceratium	*shrankii*	-	+	*Protoperidinium*	*sp2*	+	-
Ceratium	*tripos*	+	+	*Protoperidinium*	*sp3*	+	-
Ceratium	*horridum*	+	-	*Protoperidinium*	*sp4*	+	+
Ceratium	*longipes*	+	-	*Protoperidinium*	*sp5*	+	+
Ceratium	*macroceros*	-	+	*Protoperidinium*	*sp6*	-	+
Ceratium	*massilens*	-	+	*Protoperidinium*	*sp7*	-	+
Ceratocorys	*horrida*	+	+	*Protoperidinium*	*sp8*	-	+
Dinophysis	*caudata*	+	+	*Protoperidinium*	*sp9*	-	+
Dinophysis	*sp*	-	+	*Protoperidinium*	*sp10*	-	+
Euglena	*viridis*	+	+	*Pyrodinium*	*sp*	+	-
Euglena	*gracilis*	+	-	*Pyrophacus*	*steinii*	-	+
Fibrocapsa	***japonica***	-	+	*Pyrophacus*	*vancampoae*	+	-
Gonyaulax	*sp*	-	+	*Pyrophacus*	*sp*	+	-
Gymnodinium	*sp*	+	-	*Pyrophacus*	*hologium*	+	-
Gymnodinium	*fusus*	-	+	*Scripsiella*	*trochoidea*	-	+
Gyrodinium	*sp*	+	+	*Unidentified*	*sp1*	-	+
Gyrodinium	*sp*	-	+	*Unidentified*	*sp2*	+	-
Gyrodinium	*sp*	-	+	*Unidentified*	*sp3*	+	-
Gyrodinium	*sp*	-	+	*Unidentified*	*sp4*	+	-
Noctulica	*scintillans*	+	+	*Unidentified*	*sp5*	+	-
Ornithocercus	*magnificus*	-	+	*Unidentified*	*sp6*	-	+
Oxytoxum	*sp1*	-	+	*Unidentified*	*sp7*	-	+
Oxytoxum	*sp2*	-	+	*Unidentified*	*sp8*	-	+
Polykrikos	*kofoidii*	+	+	*Unidentified*	*sp9*	-	+
Prorocentrum	*compressum*	+	+	*Unidentified*	*sp10*	-	+
Prorocentrum	*sp1*	+	-	*Unidentified*	*sp11*	-	+
Prorocentrum	*sp2*	+	-	*Unidentified*	*sp12*	-	+
Prorocentrum	*lima*	+	-	*Unidentified*	*sp13*	-	+
Prorocentrum	*micans*	-	+	*Unidentified*	*sp14*	-	+
Prorocentrum	*minimum*	-	+	*Unidentified*	*sp15*	-	+
Prorocentrum	*tristinum*	-	+	*Unidentified*	*sp16*	-	+

Table 4. Dinoflagellates population in AT and AG regions. Unidentified: isolated cells < 20 ind/l.

attributed to the genus *Ceratium* with 640 ind/l at AT and 250 ind/l at AG.

As opposed to what has been noticed for toxic diatoms, the sum of toxic dinoflagellates in AG is higher than the sum in AT, respectively 860 ind/l versus 330 ind/l.

As previously mentioned, *F. japonica* is absent in AT and present AG, precisely in station 22 at 110 ind/l. The cells were rounded to oval in size 23μm × 32.8μm with multiple chloroplasts and mucocysts located in the posterior end (Fig. 6). The anterior (beating) and posterior (trailing) flagella are not apparent.

DISCUSSION

The chlorophyll and temperature values positioned at each station show that they vary inversely in the AT region (normal conditions). Indeed, there was a decreasing coast-offshore gradient of chlorophyll and an increasing gradient of temperature. While in the AG region chlorophyll and temperature vary simultaneously, noting a decreasing gradient for both chlorophyll and temperature on the 18–21 radial and an increasing gradient on the 25–22 radial, resulting in a continuous decreasing gradient from station 11 to station 25 (Fig. 7). This shape reminds us of the coastal eddies described by Millot et al. (1999) in the Algerian basin. Indeed, it can be said that the coastal gyre also exists inside the Arzew gulf and is responsible for the distribution of T °C and Chl in AG region.

We notice that the AT region is warmer than the AG region, yet AT is closer to the cold Atlantic waters entering through the Gibraltar and bordering the Algerian west coast. It is assumed that the low temperatures recorded in the Arzew gulf indicate an upwelling of coastal water.

Salinity remains relatively stable with a value of 35.5 PSU. This value correspond to the Atlantic waters that enter via Gibraltar and that border the Algerian coast (Millot et al., 1999). Nevertheless, the highest value of 36.8 PSU was recorded at station 22 where *F. japonica* was reported. On the other hand, Fani et al. (2014) found the highest abundances of *F. japonica* were recorded under salinity conditions of 36.6 PSU to 37.7 PSU.

The high presence of diatoms compared to dinoflagellates in the two regions AT and AG was also noted in the Alboran Sea by Boudjnah et al. (2019).

Nevertheless, we note that both AT and AG regions are poor in phytoplankton density, with 8870 ind/l at AT and 8150 ind/l at AG. This remark was also given by Boudjnah et al. (2019) where the lowest density of the whole Algerian coast was found in the Alboran Sea, precisely in Ain Temouchent (AT region) with 740 ind/l). Fani et al. (2014) was able to detect the presence of *F. japonica* between 1°W and 1°E and 36° and 37°N. This zone corresponds to a cyclonic eddy, which confirms its confinement in cyclonic waters only.

This zone is located off the Mediterranean Sea between Algeria and Spain, corresponding exactly to the offshore between Arzew and Cartagena. The highest cell abundance was noted at stations south of this area, near Algerian waters. In our study, *F. japonica* was detected at station 22. This station is located south of the gyre where *F. japonica* was detected by Fani et al. (2014). Nevertheless, we found a low abundance of this cell at 110 ind/l, which could be explained by the fact that Station 22 is located near and outside the hurricane zone. Furthermore, this species was not detected between 2°W and 7°W and 35° and 37°N. This zone corresponds to the western Mediterranean, from the open sea of Almeria-Oran to the Spanish Atlantic coast, including the AT region (absence of *F. japonica* in our study). Thus, the result of our study can be said to be consistent with the results of Fani et al. (2014).

Regarding the local source of *F. japonica*, Fani et al. (2014) suggests that cells of this species may have been captured in a coastal assemblage along the Spanish coast, trapped in the Almeria-Oran jet

Figure 6. Picture of *Fibrocapsa japonica* with chloroplast (c) and mucocysts (arrow).

and eventually drain offshore in cyclonic waters. Moreover, the map established by Bouda et al. (2015) of the risk level introduction species in Arzew port shows that medium and high risk category ports are located in the western Mediterranean and on the European Atlantic coast (Fig. 8). Therefore, we assume that the presence of *F. japonica* across the port of Arzew and Bethioua is of anthropogenic origin from ballast water sources from European ports (France, Spain, Italy and Germany).

CONCLUSIONS

The main biotic and abiotic factors (temperature, salinity and chlorophyll) has shown that the two regions AT and AG generally have the same

characteristics. There are, however, some small differences in values and distributions due to the different topography of the study areas and exposure to marine currents. Indeed, AT receives incoming Atlantic waters through the Strait of Gibraltar. The AG region is a sheltered gulf, the current passes outside creating an eddy inside the gult. This eddy has a major role in the distribution of the above-mentioned factors.

The study of the phythoplankton population showed a homogeneous quantitative distribution between the AT and AG regions. Diatoms and dinoflagellates have an equal density in both regions. nevertheless diatoms are more abundant in each region, and according to the results of our study and the results of the study of Boudjnah et al. (2019) it can be concluded that the dominance of diatoms
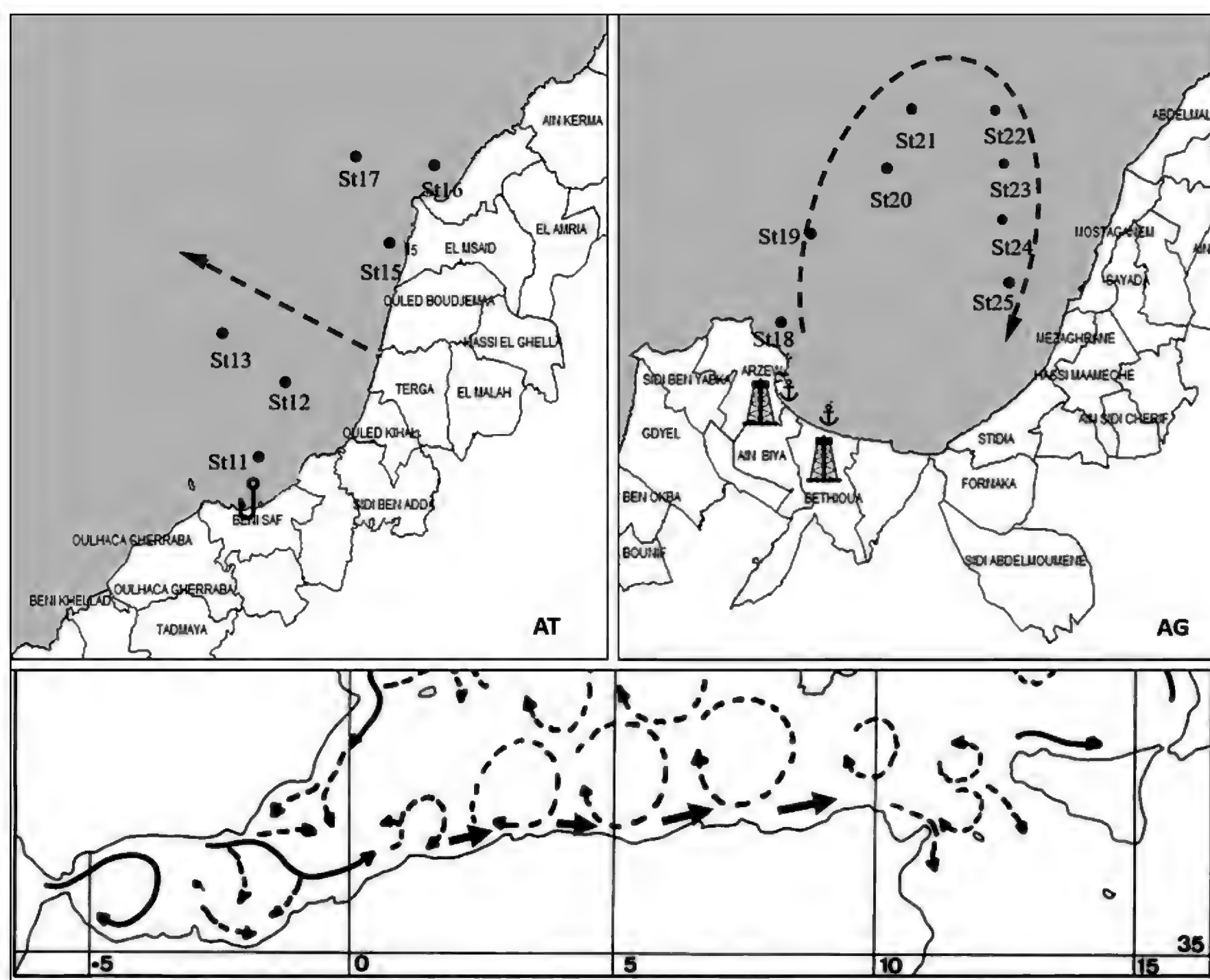
Figure 7. **Thermal and chlorophyll gradient. Bottom: map of the modified Atlantic water circulation (MAW), and the Algerian current (modified from Millot et al., 1999).**
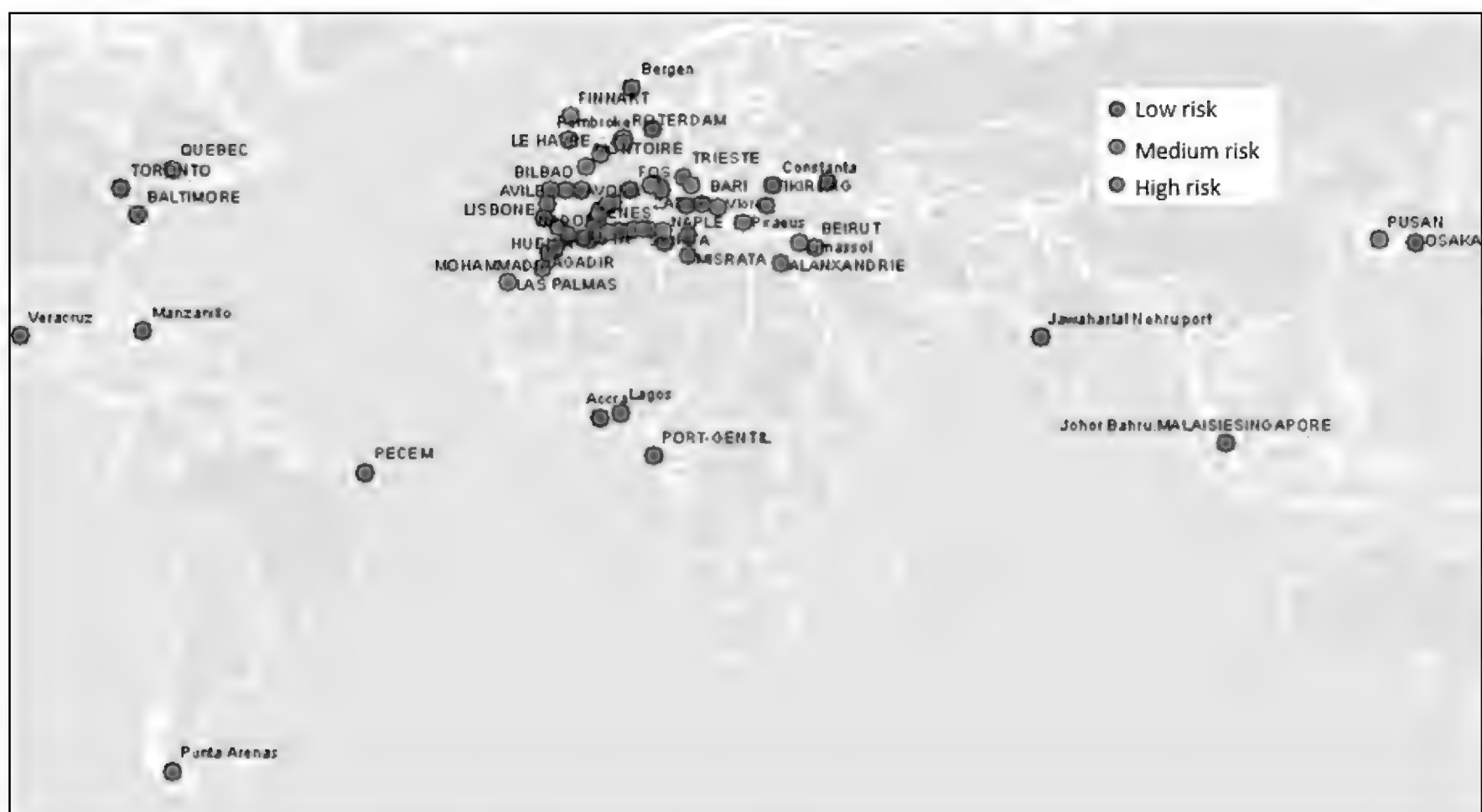
Figure 8. Map illustrating spatial patterns of all donor ports in the world,
according of their category of risk (2014) (Bouda et al., 2015).

over dinoflagellates is a characteristic of the Alboran Sea.

On the other hand, there was a qualitative difference. The most important and interesting result is the presence of the non-indigenous species *F. japonica* in Arzew Gulf (AG) and its absence in the reference area AT. We cannot really conclude the source of the presence of this species on the west Algerian coast, but it is quite possible that it is a source of ballast water, since it was found across the port of transport of hydrocarbons using ballast water for this purpose (Arzew and Bethioua), and it has not been detected in the AT zone, which has only a small fishing port.

To confirm the source of the presence of *F. japonica* in Arzew gulf, it would be best to carry out a phytoplankton analysis of seawater pumped by ships from suspected donor ports in order to identify *F. japonica* in this ballast water. It is also interesting to compare the species already present in the Arzew gulf with species of strains from the suspect regions in order to know their origin.

We notice that this species is no longer trapped in the eddy and is moving closer to the coast. However, despite its low abundance in the Gulf of Arzew, this species is still reported harmful, we fear that it

may be transported by the Algerian current to other areas of the Algerian coast, including fisheries and aquaculture production areas.

Our study is part of the prevention and conservation framework, and according to our conclusions and hypothesis, it is absolutely necessary to adopt national regulations and action programmes in order to prevent the introduction of non-indigenous species, particularly harmful ones, and to preserve local biodiversity.

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Anatomical changes of roots and steams of *Phaseolus vulgaris* L. (Fabales Fabaceae) under salinity at juvenile state

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ABSTRACT   In Algeria, the region of Mostaganem is known for its agricultural soils with a sandy tendency and abnormally loaded with soluble salts affecting the yields of crops. To assess the salt tolerance threshold of the bean culture *Phaseolus vulgaris* L. (Fabales Fabaceae) variety "coco rose" was grown in plastic pots filled with two types of substrate, sand and sand amended with 7% bentonite (calcium clay of mining origin). The test was carried out in a greenhouse with controlled climatic conditions (variant temperature between 23-25°C, humidity is around 75% and a photoperiod of 12 hours). At the 5-leaf stage, irrigation with saline was provided with four saline concentrations (0, 50, 100 and 200 meq), the control is irrigated with distilled water. Two weeks later, the microscopic observations were made with an Optica type microscope, the results show a variability of the effect of saline stress depending on the organ and the concentration of the saline treatment. The anatomical structure of the treated roots and stems has shown significant anomalies; thus, the changes are marked by the decrease in the size of the parenchymal cells, that of the diameter of the xylem vessels and the increase in their number, under the action saline concentration ($NaClCaCl_2$) and according to the type of culture substrate sand (S) and sand with bentonite (SB).

INTRODUCTION

Salinity is one of the major abiotic environmental stresses affecting about 6–7% of the world's total land area and agricultural productivity specifically in arid and semi-arid regions of the world (Qadir et al., 2006). In saline soils, high concentrations of sodium (Na) and chlorine (Cl) ions within the plant root zone retards the growth of plants by either decreasing the water potential of root media or causing toxicity of Na and Cl in various plant organs (Panta et al., 2014). Salt impose both osmotic and ionic stresses on the plants which lead to several morphological and physiological changes (Jampeetong & Brix, 2009). A clear stunting of plants is noticed due to salinity stress (Takemura et al., 2002). High salt content, especially chloride and sodium sulphates, affects plant growth by modifying their morphology and anatomy (Huang & Redmann, 1995; Dolatabadian et al., 2011). The effect of salinity on root (An et al., 2003) and shoot anatomy (Ali et al., 1999) of plants had already been reported in previous works. Many researchers reported that with an increase in salinity there was a decrease in the development of the xylem. Pimmongkol et al. (2002) stated that the width of vascular bundles and

diameters of rice stems decreased in NaCl medium. The earliest response is a reduction in the rate of expansion of the leaf surface, followed by cessation of expansion as stress increases (Parida & Das, 2005). Munns & Tester (2008) reported that salt-sensitive plants have reduced survival, growth and development when exposed to even low to moderate salinities, while salt-tolerant species are able to grow and reproduce in saline environments.

The aim of this work is to study the effect of salinity not only at the scale of different soils, but also on the mechanisms of response of the plant *Phaseolus vulgaris* L. (Fabales Fabaceae) at different concentrations of $NaClCaCl_2$ on sandy soil with or without bentonite. An anatomical study of stems and roots, microscopic visualization and examination of possible correlations between anatomical changes and salinity levels were done.

MATERIAL AND METHODS

Plant materials and culture mode

Phaseolus vulgaris L. coco rose variety is used as plant material for this experience.

The crop is grown in two different types of substrate: sand and sand mixed with 7% of bentonite clay mineral of calcium origin). The substrate is placed in plastic pots of 15 cm diameter and 20 cm height with a capacity of 2 kg.

Preparation of the culture substrate

The sand is washed beforehand with dilute hydrochloric acid N, rinsed thoroughly with distilled water to remove the chlorides and dried in an oven at 105°C. Thus prepared, the sand constitutes a support of the plant (1 plant per pot), allows aeration of the roots and has the advantage of not fixing the ions. After natural drying, the bentonite is crushed then mixed with the sand with a dose of 7%. The Sand-Bentonite mixture is carefully homogenized manually, then filled into the pots. Beforehand the bottom of the pots is lined with a layer of one cm thick gravel of 0.5 cm in diameter serving as drains. On this layer is deposited a gas strip to retain the sand. *Phaseolus* seedlings were grown for 1 month, and watering with nutrient solution Hoagland & Arnon (1938). The saline solution consists of two

salts NaCl and $CaCl_2$ concentrations (50, 100, and 200 meq), the control is sprayed with the nutrient solution. The stress is applied at the 5-leaf stage and then repeated at a frequency of one intake per three days for three weeks.

Anatomical study of stems and roots

After each treatment, one seedling per pot is dug up and stripped of the substrate by rinsing with distilled water. This operation is repeated three times per treatment. The organs (stems and roots) are carefully separated by means of a razor blade and then cut into pieces 1 to 2 cm long. Only the samples of the median parts are taken into consideration. Cross sections are performed "freehand" on stems and roots by means of a razor blade. Thin sections with a thickness of 20 μm are stained by the double staining technique (methyl green/Congo red). The sections are first treated with 8% sodium hypochlorite for 15 minutes. After careful rinsing with distilled water, they are etched with dilute 70% acetic acid for 2 minutes and then stained with 1% methyl green for 5 minutes; the latter colors the lignified walls in green. The pieces are then washed with distilled water and stained with 2% Congo red for 15 minutes. This dye highlights the cellulose that appears in pink or red.

Double staining technique

The sections are then washed with distilled water and mounted in a drop of water between the slide and coverslip before being observed first under ordinary microscope, then on another microscope allowing good shooting and taking pictures. The cups are kept either in pillboxes containing distilled water or in a drop of Canada balm placed between blade and coverslip. Once the sections are stained, they are observed by a microscope of the type Optica menu of apparatus of high definition in taking microscopic photo; a photo tube used for taking micrographs.

Statistical analysis

An experimental design of plots in complete random block was carried out using 3 plants per treatment. Three roots and three stems per plant were sampled for anatomical studies. Data were an-

alyzed by ANOVA using the Newman-Keuls Least Significant Difference Test (LSD) for mean comparisons using a significance level of 5%.

RESULTS

The sections obtained, used as experimental material under observation under a microscope, made it possible to measure the cells of conducting vessels specifically the root and shoot xylem. These measurements concerned measurements of the diameter of the vessels using a micrometer adapted to the microscope.

To show the effect of salinity on the evolution of the root diameter of the cylinder vessels of the bean variety tested, observations under a microscope are made on seedlings grown in substrate without bentonite (Fig. 1). The results obtained show a root cylinder diameter of (27.2 µm) in the control, this diameter decreases significantly under the effect of salinity to reach (8.77 µm) at 200 meq of $NaClCaCl_2$ (Table 1). Observations using the microscope at magnification (X40), the photos in the figure 3 (a, b, c and d) clearly show a very significant reduction in the diameter of the vessels of the root cylinder under the salinity effect.

The anatomy of the stem gives us structural changes in the cylinder elements. After 21 days of growth in the substrate without bentonite, the bean seedlings stressed with $NaClCaCl_2$ register diameters of the cylinder cells substantially identical in the control and at 50 meq around 20 µm. As soon as the salinity increases in the culture medium, the measurements made on the cells of the stem cylinder give a diameter which decreases sharply to reach (13.5 µm) at 100 meq, then (8.9 µm) at 200 meq (Table 2). These observations are confirmed with the anatomical study; thus, anatomical sections are made on the stems of bean seedlings aged 21 days grown in sand alone and stressed with $NaClCaCl_2$. Microscopic observations at magnification (X40) show variations in the number of cylinder vessel cells (Fig. 2). This explains why, when the salinity increases in the culture medium, the number of cells per vessel decreases as well as their diameter.

As shown in figure 3, results indicate the influence of salinity on the variation of the root xylem diameter of the vessels. After 21 days of growth, the

roots of the bean variety have a maximum diameter of the xylem vessels with (27.2 and 28.13 µm) by the control, on the other hand, at the 200 meq of $NaClCaCl_2$, these diameters decrease to give a minimum of 8.83 and 17.03 µm grown respectively on S and SB substrate. The sand amended with bentonite made it possible to display larger xylem diameters than those of the substrate without bentonite with 23.5 and 19.37 µm against 17.67 and 13.43 µm respectively at 50 meq and 100 meq of $NaClCaCl_2$. Statistical calculations show that
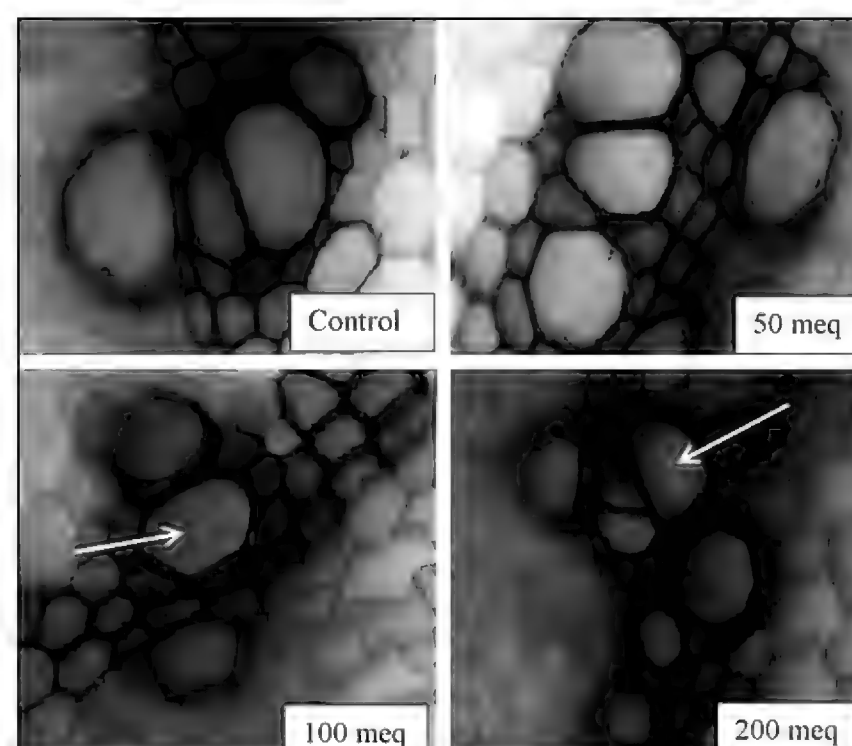
Figure 1. Root anatomy of *Phaseolus vulgaris* L. variety "coco rose", 21 days old. The arrows indicate the xylem vessels (magnificationX40).
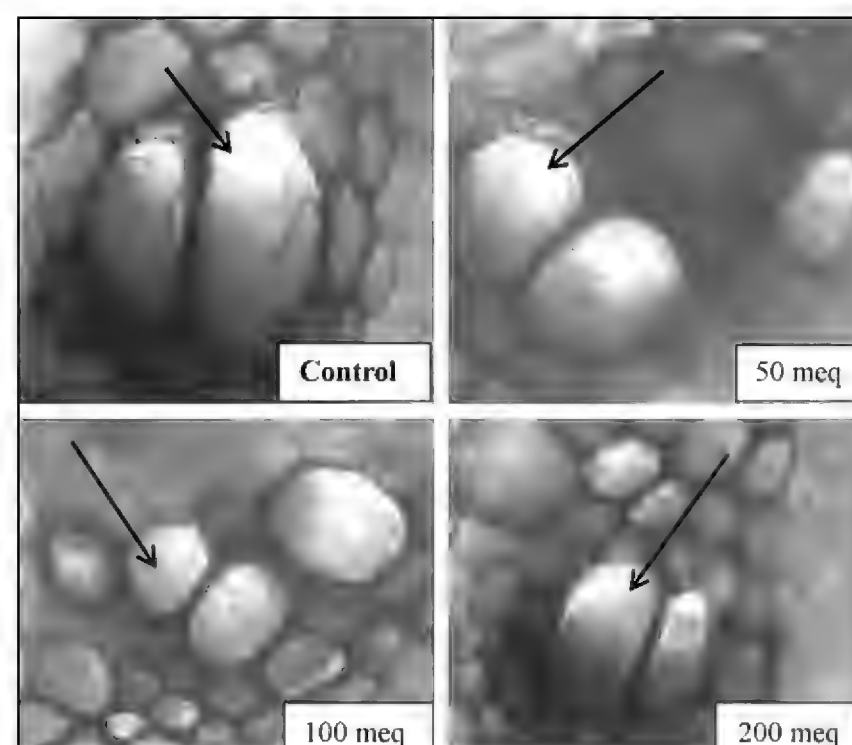
Figure 2. Stem anatomy of *Phaseolus vulgaris* L. variety "coco rose", 21 days old. The arrows indicate the xylem vessels (magnificationX40).

Substrate	Control	50 meq	100 meq	200 meq
WOB ***	27.2 [a ns]	17.67 [b]	13.43 [d]	8.77 [f]
WB***	28.13 [a ns]	23.5 [c]	19.37 [e]	17.03 [g]

Table 1. Effect of salinity and substrate on root cylinder diameter (μm) of *Phaseolus vulgaris* L. Means in each row followed by different letters are significantly different (p <0.05). *, **, ***: Differences between substrate is significant at P < 0.05, 0.01, and 0.001 respectively, ns: not significant at (P< 0.05).

Substrate	Control	50 meq	100 meq	200 meq
WOB ***	20.9 [a]	18.5 [a]	13.5 [b]	8.9 [d]
WB***	22.9 [a]	21.4 [a]	19.3 [c]	16.1 [e]

Table 2. Effect of salinity and substrate on stem cylinder diameter (μm) of *Phaseolus vulgaris* L. Means in each row followed by different letters are significantly different (p <0.05). *, **, ***: Differences between substrate is significant at P < 0.05, 0.01, and 0.001 respectively, ns: not significant at (P< 0.05). Interaction between substrate and NaClCaCl2 is not significant at P<0.05.

there is a very significant effect of salinity on the diameter of root xylem diameter, the sand culture substrate amended with bentonite records a very significant effect compared to the sand substrate alone (p <0.05) (Table 1).

After 21 days of culture (Fig. 4), the bean plants from S and SB respectively register a maximum diameter of the stem cylinder with 20.9 and 22.9 μm by the control, on the other hand, the minimum is recorded at 200 meq of $NaClCaCl_2$ with 8.9 and 16.1 μm. Overall, the sand culture substrate improved with bentonite has larger shoot diameters with 21.4 and 19.3 μm than those of the S with 18.5 and 13.5 μm respectively under salinity with 50 and 100 meq of $NaClCaCl_2$. Statistical calculations show that there is a very significant effect of salinity on the stem cylinder diameter. The SB has a significant effect at 100 and 200 meq. However, the interaction between substrate and $NaClCaCl_2$ is not significant at P<0.05 (Table 2).

DISCUSSION

The salinity induced structural changes in the xylem of the stems and roots. In plants stressed with $NaClCaCl_2$, the thickness of the shoot vascular cells was much higher than in the control; the effect of salinity depended on the salt concentration and the growing medium. Al-Tardeh & Iraki (2013) studied the seedlings of two varieties of tomatoes exposed to salt stress and concluded that salinity reduces root vascular function. In addition, the profiles of the phloem and xylem parenchyma were significantly reduced in saline environments. Other studies confirm that the salinity of the environment modifies the anatomical structure of the root and leads to a decrease in the number of cells per xylem bundle and the number of layers of cortical parenchyma (Haouala et al., 2007; Farhana et al., 2014). In general, plants grown in bentonite-modified soil had a larger xylem vessel diameter than those grown in sand without bentonite, this is likely due to the role of calcium-rich bentonite in mitigating the effect of salinity, as pointed out by Hellal et al. (2015). These results were approved by Arbaoui (2016), with 10% bentonite in sandy soils, the effect of salinity is reduced on tomato plants. Saline stress is associated with a greater deposit of lignin in the vascular tissue and/or the development of xylem. $NaClCaCl_2$ causes significant lignifica-
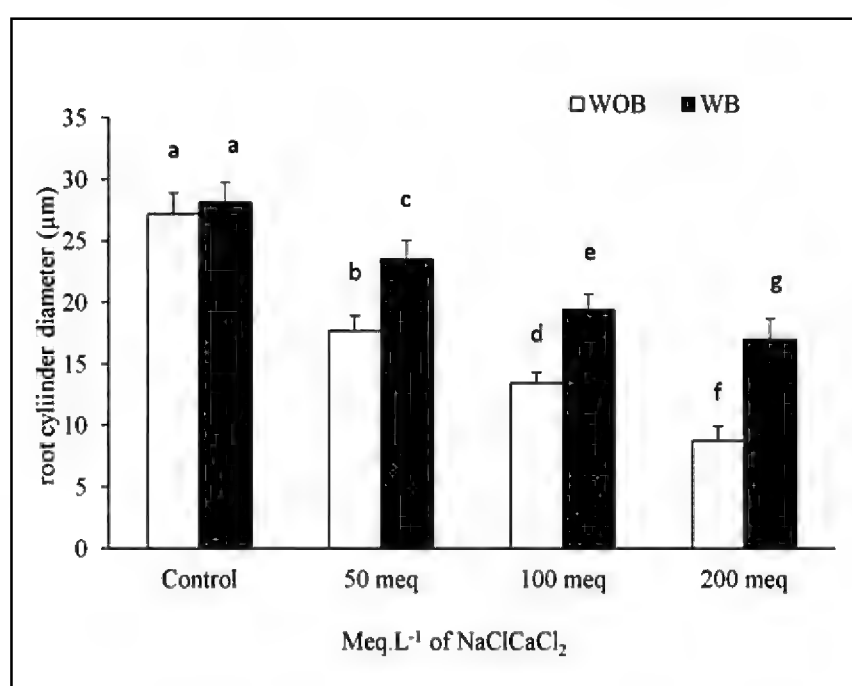
Figure 3. Root cylinder diameter (μm) after 21 days of bean variety "coco rose" cultivated on substrate (S and SB) stressed with NaClCaCl2. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at p<0.05. The same letters show no significantly different values.
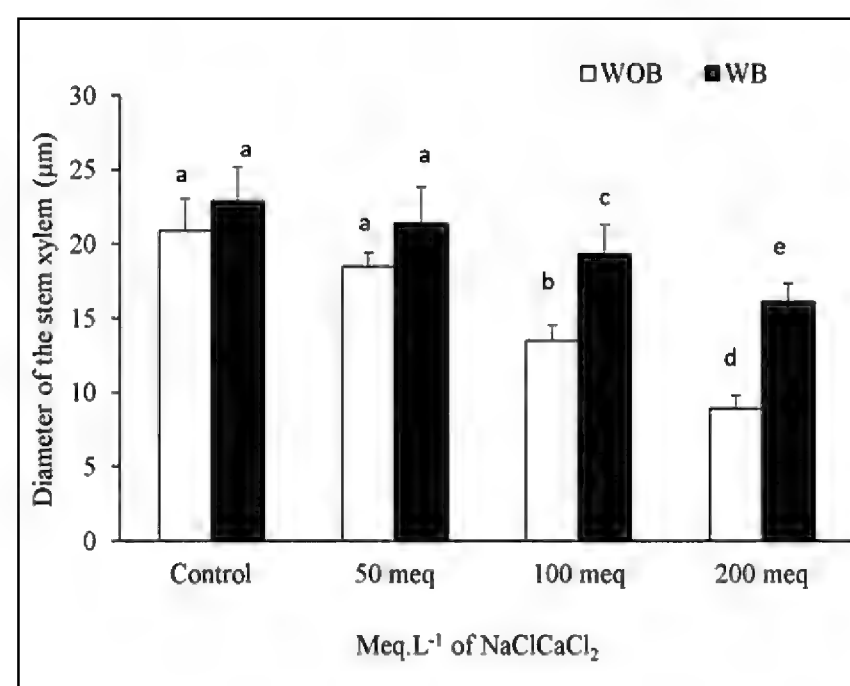
Figure. 4. Stem cylinder diameter (μm) after 21 days of bean variety "coco rose" cultivated on substrate (S and SB) stressed with NaClCaCl2. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at p<0.05. The same letters show no significantly different values.

tion in root and caular vascular tissues, suggesting a factor that inhibits root growth and, therefore, represents an adaptation mechanism to resist the stress imposed by salinity (Cachorro et al., 1993). Vascular tissue and the size of plant cells are reduced when exposed to salinity. In addition, the cross-sectional areas of the roots of plants exposed to salinity were considerably reduced, so that the roots of salt-stressed seedlings exhibited reduced vascular function and cortical parenchyma compared to control plants. These changes in the number and diameter of xylem vessels have had a significant impact on water consumption and transport according to several authors (Choat et al., 2005; Alsafary et al., 2019). According to several studies, the adaptation of plants to salt stress is accompanied by physiological changes (Shannon, 1997) and anatomical changes (Hwang & Chen, 1995; Çavuşoğlu et al., 2007), inhibition in diameter and number of xylem. Introduced by Kiliç et al. (2007), so salinity stress induced the production of new protein bands do not occur in the control plants (see also Dawood & El-Awadi, 2015). In addition, the salt response of plant species depends on several variables, starting with the species itself, its variety, salt concentration, growing conditions and stage of plant development (Bennaceur et al., 2001; Alaoui et al., 2013). The identification of salt tolerant varieties and genotypes, capable of mini-

mizing the depressive effects of salinity on yields, would certainly improve agricultural production in salinity-affected areas. In non-Halophytes, there is great variability in responses of sensitive or salt stress tolerant species based on the lipid composition of the roots (Greeway, 1980). The effect of salinity on the lipid composition of roots has been studied in different species, including grapes, bean and plantago (Erdel et al., 1980). However, the mechanism of adaptation of plants to salinity is not fully known. Calcium plays a crucial role in the stabilization of cell membranes. Also, it is known to have an improving effect on plant growth stress (Hyder & Greenway, 1965; Deo & Kanwer, 1969; La Haye & Epstein, 1971).

CONCLUSIONS

The presence of calcium in bentonite and in the solution of irrigation has played an important role in reducing the effect of salinity. This is due to the contribution of bentonite and the mixture of NaCl with $CaCl_2$ that made it possible to improve the number of cells of the vessels of the xylem, whether it is the root or the stem. Finally, an addition of 7% bentonite to sandy soils with a saline tendency improves the structure of sandy soils.

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Temporal variation of bird biodiversity and compositional complexity in a representative semi-Agricultural Natura 2000 area of conservation in Northern Greece

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ABSTRACT   In this work we study the alteration in compositional diversity of bird abundances at the species level from 2012 to 2017 in one of the most important wetland Natura sites in Northern Greece and by using different biodiversity indices. Shannon Entropy was lower during 2012 (D_H = 1.509) albeit remained in similar levels from 2013 and afterwards. The highest values of Shannon Entropy were recorded in 2014 (D_H = 2.927) and 2016 (D_H = 2.888) suggesting that there is a higher diversity compared to the other observation years and especially 2012. The yearly trends of the Simpson dominance index and the Gini-Simpson Index had quite similar patterns. The Berger-Parker index, D_D, which represents the maximum proportion of any species estimated in the sample assemblage, had its highest values in 2012 (D_D = 0.58) and 2017 (D_D = 0.39) and its lowest in 2014 (D_D = 0.13) and 2016 (D_D = 0.15). A complete characterization of diversity was possible through the projection of Hill numbers and the Rényi entropy, parameterized by the order q in terms of an empirical curve. According to the Hills numbers pooled over the years, the mean species abundance (q = 0) was estimated at 31 species, the mean biodiversity (q = 1) was 13 species and the most dominant species (q = 2) were 8 species. The quantification of bird biodiversity in the particular research area patterns is a fundamental task to evaluate current management actions, improve conservation and design future management strategies.

KEY WORDS   Birds; compositional complexity; biodiversity indices..

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INTRODUCTION

NATURA 2000 network is the main pillar of the European Union for the conservation of species biodiversity and the protection of the natural environment. The operation and configuration of the Natura 2000 network is based on two fundamental EU directives, the habitats directive (EU Habitats Direc-

tive: 92/43/EEC 1992)) and the Birds directive (EU Birds directive 2009/147/EC). According to theses directives the member states must assure that habitats are not degraded or disturbed, and monitor the condition of the protected species and habitats. Since the Natura network does not exclude human economic activities, such as farming, fishing, etc., it is quite important to have quantitative measures that can be used to evaluate the compatibility of

human activities with protection of valuable species and habitats.

Quantitative biodiversity indicators include information that describe the biodiversity of species in different scales (Austin 1983). At a simplest level, the number of species in a community is a measure which referred as species richness or alpha diversity. However, species richness is a rather simple numerical count of the number of different types of species present since it does not takes into account species evenness which provides fundamental information on the uniformity of a species. Therefore, the use of more straightforward biodiversity (i.e., Shannon-Weaver), which combine the effects of richness and evenness, putting more or less weight to each case, provide a robust measure of species biodiversity (Maguran, 1988, 2004).

Because birds are very sensitive to habitat and environmental changes, they are therefore considered as one of the best indicators to monitor species biodiversity and provide vital information on the possible changes that are expected to occur in the future ecosystems (Niemi et al., 2004; Pan-European Common Bird Monitoring Scheme, 2006). In addition, because the extension of agricultural land and intensification of agriculture is widely recognized as a major cause of declining farmland bird populations, there is an ongoing interest in studying how bird biodiversity is affected during the past years by climate and human activities (Wilson, 1989; Malkolm et al., 2006; Sekercioglu et al., 2008; Guerrero et al., 2012).

The Thermaikos Gulf protected area is part of the European Natura 2000 ecological network of protected areas (Figs. 1, 2) and among the most important Natura cites in Greece offering major ecosystem services throughout many years (Karageorgis et al., 2006; Vareltizidou & Stinxer, 2009; Panagiotopoulou et al., 2012). The largest part of this protected area has been listed as a National Park, through Joint Ministerial Decision (JMD) 12966/2009 and includes the deltas and the estuaries of four rivers, the Lagoon of Kalochori and the Alykes Kitrous, the wetland of Nea Agathoupoli and the riverbed of Axios, reaching until the Elli dam (Axios Delta Management Authority, 2013). Although more than 290 bird species have been recorded in this area, of which some are globally threatened, current treats makes the management of the Thermaikos Gulf protected area a challenging

goal (Kazantzidiz & Goutner, 2008; Vokou et al., 2018).

The aim of the current work was the study of some representative bird population in Northern Greece and how they change throughout the years using straightforward biodiversity indices that combine the effects of species richness and evenness. Moreover, since species abundance does not capture all information on species diversity, the scope was to provide for the first time a complete characterization of the compositional complexity of bird assemblages. Moreover, because measuring actual bird biodiversity is not an easy task the use of different summary statistics and related biodiversity indexes consist of a robust tool that accurately describe the trends in components of biodiversity under certain habitat conditions and can be further used as an important tool for decision makers (Purvis & Hector, 2000; Gregory, 2006).

Therefore, in order to quantify the differences on bird species community, the current study focus on the estimation of different biodiversity indices. Multiple statistical strategies and formulas are used to figure out which birds have declined or increased over time and to compare year to year variations in bird biodiversity. Proposing different statistical biodiversity indices, as well as measuring related changes in bird biodiversity, provide new information that is particular useful in evaluating conservation and habitat management actions. This is particularly important concerning that a big part of the protected area consist of agricultural cites. Additionally, positive or negative changes in the biodiversity indices over time may provide information on priority species and may be used to judge whether to modify or establishing new bird conservation plans (BCPs).

MATERIAL AND METHODS

Bird data and study area

For the current biodiversity study, we have used bird data of the Natura 2000 network that are freely accessible in the internet and published in the official website of the Thermaicos gulf protected areas management authority (Theramaikos gulf protected areas management authority, 2020). In particular, the data refer to monthly records of the number of bird
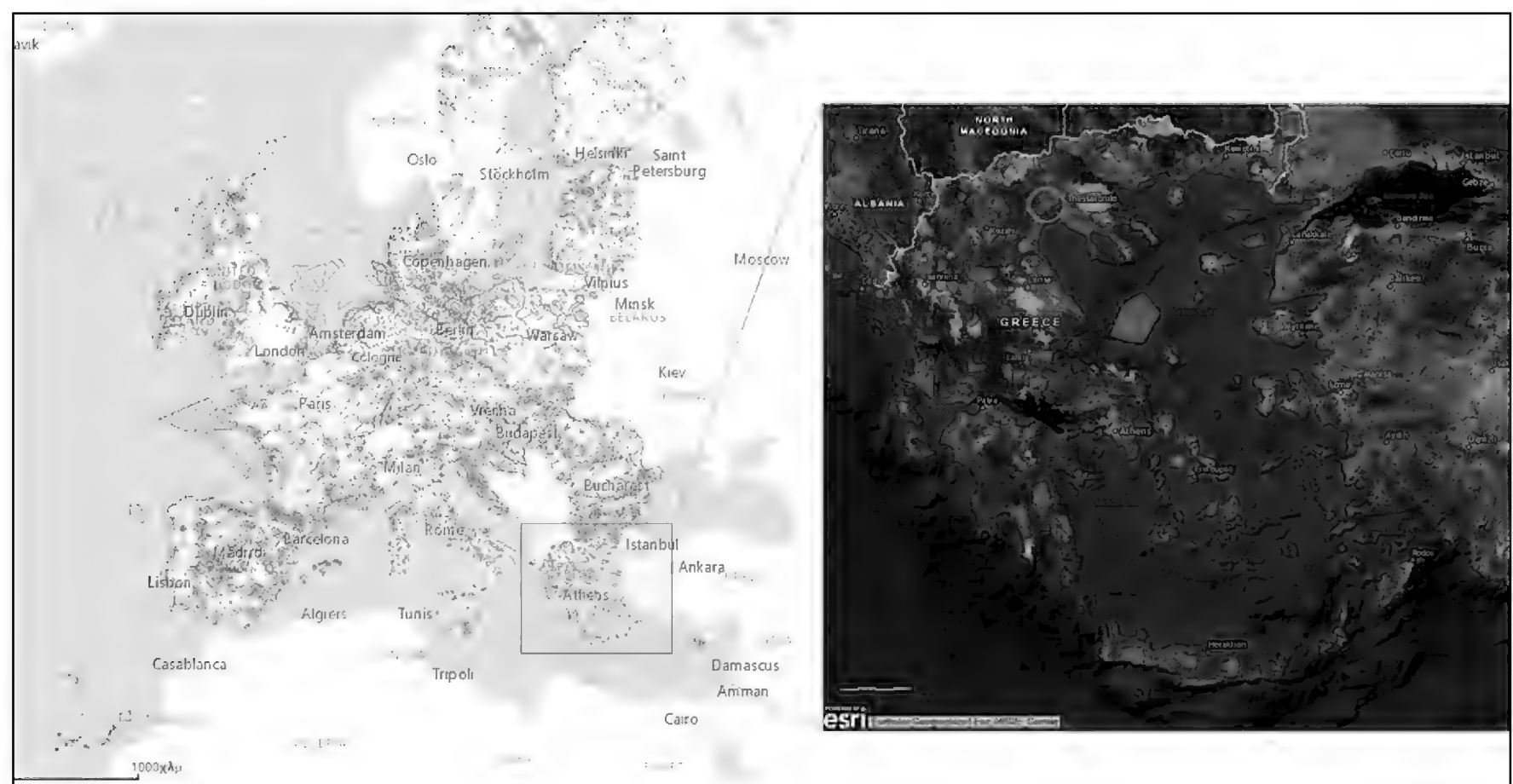
Figure 1. European Natura 2000 ecological network of protected areas (left) and the Natura 2000 ecological network in Greece (right) designed according to the Birds Directive (Spatial protection areas) and the Habitats Directive (Sites of Community Importance, and special areas of conservation). Green areas indicate spatial borders of sites, Pink areas: special protection areas of birds and blue areas zone of special protection of birds. The orange circle points the area were the research is performed (free maps provided by the EU Natura 2000 network public viewer and Esri earthstar geographics).

Figure 2. The Natura 2000 National Park of the Delta of Axios - Loudias - Aliakmon on the west coast of the Thermaic gulf in Northern Greece. The Green shadowed area represents the borders of the protected area including the delta created by three rivers Aliakmonas (red line), Loudias (pink line) and Axios (yellow line) (free map provided by Esri earthstar geographics).

species, from 2012 until 2017, from inside the spatial protection zone (GR1220010) of the Natura 2000 natural park which is located in the west coast of the Thermaic gulf in Northern Greece (Fig. 1). In addition, since there were instances in which some observations were absent (mostly due to technical reasons), the most complete and representative bird watching data sets from 2012 until 2017 were used.

The special geography and climatic conditions favor the development and evolution of a great diversity of bird populations. In particular, the location lies close to the coast and consist of a wetland complex which includes the Lagoon of Kalochori, the estuary of the Gallikos river, the delta of the Axios river, the estuary of the Loudias river, the delta of the Aliakmon river, the wetland of Nea Agathoupoli and the Alyki Citrus wetlands as well as closely related agricultural and semi urban areas (Fig. 2).

The climate of this area is classified as Csa Mediterranean climatic type (Köppen, 1923; Lochmann et al., 1993) with long, hot, and dry summers (the mean maximum temperature lies often in the range of 29.0 and 35.0 degrees of Celsius), relatively mild and rainy winters, and average annual air temperatures of approximately 15°C. To date, 299 bird species have been documented in the above area and represent close to 66% of all bird species observed in Greece (Theramaikos gulf protected areas management authority, 2020). All data downloaded from the internet are intellectual property of the Thermaicos gulf protected areas management authority and have been used after permission for the particular research of the University of Alicante, Spain (license permission: Chalastra 19/3/2019, protocol number: 262).

Biodiversity indices

The biodiversity indexes are mathematical expressions which measure the species diversity in a community (Maguran 1988, 2004). The major advantage of biodiversity indices, compared to simply species richeness (i.e., number of species present), is the fact that they take in to account the relative abundance of different species and therefore, they provide important information on the species community structure. Since biodiversity indices enclose certain details and expression about the commonness and rarity of a species in a loca-

tion and/or time of observation (Roth et al., 1994; Rosenzweig, 1995) numerous indices were taken into account.

In particular, for the present study the following diversity indices were estimated (Colwell, 2009):

Species richness

The species richness, D_S, refers to the total number of species, n_i, recorded in an ecological community, landscape or region (i.e., birds observed from a monitoring point). Thus:

$$(1)\ D_S = \sum n_i$$

Note that S does not take in to account abundances of the species but simply count the species found in a sample (Colwell, 2009).

Shannon Entropy

The Shannon diversity function, D_H, is a measure of the information necessary to specify an assemblage and probably the most common biodiversity measure. Shannon diversity is:

$$(2)\ D_H = -\sum_{i=1}^{S} p_i \ln p_i$$

Where S is the number of species and p_i is the relative abundance of species i (i.e., the number of species n_i divided by the total number of individuals N in that community, or: $p_i = n_i/N$). For an equal distribution, all species in the data set are equally common, the Shannon entropy has the value of the natural logarithm of Richness:

$$(3)\ D_{H\max} = \ln(D_S)$$

The physical-ecological interpretation of equations (2) and (3) is that the more unequal the proportional abundances of species, the smaller the Shannon entropy. For only one type in the data set, Shannon entropy equals zero. Therefore, high Shannon entropy stands for high diversity and low Shannon entropy for low diversity.

Shannon's equitability

The Shannon's equitability is simply the Shannon diversity index divided by the maximum diversity:

$$E_H = D_H/D_{H\max}$$

Simpson diversity and dominance

The Simpson's diversity index (Simson, 1949),

D, is a simple mathematical measure that characterizes the species diversity in a community and is:

$$D = -\sum_{i=1}^{S} p^2_i$$

In addition, the reciprocal of Simson index is often used in the same context and referred as Simson dominance index:

$$(4)\ D' = 1 / \sum_{i=1}^{S} p^2_i$$

where S is the number of species and p_i is the relative abundance of species i (i.e., the number of species divided by the total number of individuals in that community).

(Gini) Simpson's dominance (evenness) index

The Simpson's dominance index, $\bar{D}$, is related to the usually Simpon's Diversity index, D, as follows:

$$(5)\ \bar{D} = 1 - D$$

$\bar{D}$ corresponds to true probability value and therefore this metric ranges from 0 (perfectly uneven) to 1 (perfectly even) (Guiasu & Guiasu, 2012). According to Hulbert (1971), the Simpson evenness stands for the probability of interspecific encounter between two specimens, which are picked randomly from a sample. Moreover, Simpson's evenness is mathematically closely related to rarefaction estimates of species richness.

Berger-Parker Index

The Berger-Parker index, (referred as species dominance in Berger and Parker 1970) is the maximum proportion of any species in a sample:

$$(6)\ D_D = \max p_i$$

If the community (sample) is dominated by the most common species and it is not even then The Berger-Parker index should be high.

Hill numbers and complete characterization of the species diversity

Hill numbers, q, or the effective number of species, are increasingly used to characterize the taxonomic, phylogenetic, or functional diversity, ^qD, of an assemblages (Chao et al., 2014; Chao & Jost, 2015). In the current study the first four Hill numbers are also estimated for comparative reasons, namely: $q = 0$ (species richness), $q = 1$ (the exponential of Shannon's entropy index), $q = 2$ (the inverse of Simspon's concertation index).

Hill numbers (Hill, 1973), consist of a class of diversity measures which integrates species richness and species abundances as follows:

$$^qD_y = (\sum_{i=1}^{S} p^q_i)^{1/(1-q)}, q \# 1$$

Where S is the number of species in an assemblage, p_i is the relative abundance of the ith species and q is a parameters which determines the sensitivity of the measure to relative frequencies. For q=0, then ^0D is simply the species richness. For q=1, $^1D = \lim (n\rightarrow1)\ ^qD_y = \exp (-\sum_{i=1}^{S} pi\ lnpi)$

Which is the exponential of the Shannon index which weights the species in proportion to the frequency.

For q=2, $^2D = \sum_{i=1}^{S} pi$

Which is the Simson's diversity index.

The complete characterization of the species assemblages is estimated also for q = 3, q = 4 and for q = ∞ and further conveyed by a diversity plot generated for each observation year.

Rényi entropy and generalized biodiversity dimensions

The Rényi entropy, H_a (p) is a generalization of Shannon's entropy. H_q (p) of order q ∈ [0, ∞] is estimated as:

$$H_q (p) = 1/1\text{-}a \ln \sum_{i=1}^{S} p^qi$$

where q is a parameter that modulates the index's sensitivity to species abundances. Shannon entropy is the limiting instance of Rényi entropy as q→1 (Daly et al., 2018).

For the given bird abundance data set from 2012 throughout 2017 these complexity measures are plotted as a continuous empirical function of q to capture the changes of the single bird assemblage over the different time periods.

RESULTS

Bird species records in the Theramaikos gulf Natura 2000 protected area

The Thermaikos gulf protected area has a rich diversity of birds. According to the review on the available data of the spatial protection zone (GR1220010), which are published by the Natura 2000 management body, a total of 71 species were recorded from 2012 until 2017. These include the

species shown in Table 1 (Theramaikos gulf protected areas management authority, 2020). Among them the most predominant species were: *Ergetta garzetta* (470 individuals), *Himantopus himantopus* (592 individuals), *Philomachus pugnax* (353 individuals), *Tringa sp* (493 individuals) (Fig. 3).

Yearly trends of bird community's biodiversity indices

Figure 4 depicts the annual changes of the bird biodiversity indices which were calculated using available data from 2012 until 2017. Shannon Entropy was

Accipiter nisus (Accipitridae, bird of prey)	*Actitis hypoleucos* (Scolopacidae)	*Anas clypeata* (Anatidae, Water bird)
Anas crecca (Anatidae, water bird)	*Anas querquedula* (Anatidae, migratory water bird)	*Anas penelope* (Anatidae, Water bird)
Anas platyrhynchos (Anatidae, water bird)	*Anthus campestris* (Motacillidae, migratory)	*Ardea cinerea* (Ardeidae, Predatory wading bird)
Ardea purpurea (Ardeidae, water bird), *Botaurus stellaris* (Ardeidae, water bird), *Burhinus oedicnemus* (Burhinidae water bird)	*Ardeola ralloides* (Ardeidae, migratory bird), *Buteo buteo* (Accipitridae, bird of prey), *Calidris alpine* (Scolopacidae, migratory bird)	*Athene noctua* (Strigidae bird of prey) *Buteo rufinus* (Accipitridae bird of prey), *Calidris ferruginea* (Scolopacidae, migratory wading bird)
Calidris minuta (Scolopacidae, migratory)	*Calidris* sp. (Scolopacidae, migratory bird)	*Casmerodius albus* (Ardeidae, migratory wading bird)
Charadrius alexandrines (Charadriidae, water bird)	*Charadrius dubius* (Charadriidae, migratory bird)	*Chlidonias leucopterus* (Laridae, migratory)
Chroicocephalus gene (Laridae, migratory bird)	*Ciconia ciconia* (Ciconiidae, migratory bird)	*Ciconia nigra* (Ciconiidae)
Circaetus gallicus (Accipitridae, bird of prey)	*Circus aeruginosus* (Accipitridae, bird of prey)	*Circus cyaneus* (Accipitridae, bird of prey)
Circus pygargus (Accipitridae, migratory bird of prey)	*Coracias garrulous* (Coraciidae)	*Cygnus olor* (Anatidae, swan)
Emberiza melanocephala (Emberizidae, migratory)	*Egretta garzetta* (Ardeidae, migratory bird)	*Ergetta alba* (Ardeidae migratory wading bird)
Falco tinnunculus (Falconidae, bird of prey)	*Fulica atra* (Rallidae, vagrant)	*Gallinula chloropus* (Rallidae, migratory water bird)
Gallinago gallinago (Scolopacidae)	*Gelochelidon nilotica* (Laridae, migratory	*Glareola pratincola* (Glareolidae, migratory)
Himantopus himantopus (Recurvirostridae, water bird)	*Hydrocoloeus minutus* (Laridae, vagrant)	*Larus genei* (Laridae, vagrant)
Larus melanocephalus (Laridae, water bird)	*Larus michahellis* (Laridae, migrant)	*Larus ridibundus* (Laridae, migratory)
Melanocorypha calandra (Alaudidae)	*Motacilla alba* (Motacillidae, migratory)	*Motacilla flava* (Motacillidae, migratory)
Nycticorax nycticorax (Ardeidae, migratory)	*Numenius arquata* (Scolopacidae, migratory)	*Phalacrocorax carbo* (Phalacrocoracidae, migratory wading bird)
Phalacrocorax pygmeus (Phalacrocoracidae, partially migratory)	*Philomachus pugnax* (Scolopacidae, migratory)	*Phoenicopterus roseus* (Phoenicopteridae, migratory wading bird)
Phoenicopterus ruber (Phoenicopteridae, wading bird)	*Platalea leucorodia* (Threskiornithidae, migratory)	*Plegadis falcinellus* (Threskiornithidae, migratory wading bird)
Sternula albifrons (Laridae, migratory seabird)	*Sterna nilocita* (Gelochelidon, migratory)	*Tachybaptus ruficollis* (Threskiornithidae, migratory wading bird)
Tadorna tadorna (Anatidae)	*Tringa* sp. (Scolopacidae)	*Tringa glareola* (Scolopacidae, migratory)
Tringa nebularia (Scolopacidae, migratory, wading bird)	*Tringa tetanus* (Scolopacidae)	*Upupa epops* (Upupidae)
Vanellus spinosus (Charadriidae, wading bird)	*Vanellus vanellus* (Charadriidae, migratory)	

Table 1. Bird species (family, and basic ecological characteristics) registered by the management authority in the spatial protection zone (GR1220010) of the Natura 2000 National Park of the Delta of Axios - Loudias - Aliakmon in Northern Greece from 2012 until 2017.

lower during 2012 (D_H = 1.509) albeit remained in similar levels from 2013 and afterwards. The highest values of Shannon Entropy were recorded in 2014 (D_H = 2.927) and 2016 (D_H = 2.888) suggesting that there is a higher diversity compared to the other observation years and especially 2012.

Shannon's equitability index, E_H, showed similar patterns and was respectively higher in 2014 (E_H = 0.85) and 2016 (E_H = 0.79) compared to all other years of observation and especially 2012 which had its lowest values (E_H = 0.46). This was expected considering that E_H is the product of the Shannon diversity index divided by the maximum diversity and normalizes D_H to a value between 0 and 1 (Fig. 3). The higher values of E_H calculated in 2014 and 2016, respectively, represent virtually a very high equitability of species which is quite close to complete species evenness.

The yearly trends in Simpson dominance index, D', in respect to year are shown in figure 5. Because D' measures the probability that two individuals randomly selected from a sample belong to the same species, values close to 0 represent infinite diversity and values close to 1, no diversity at all. The lowest values of this index were calculated in 2014 (D' = 0.06) and in 2016 (D' = 0.08), whilst the highest in 2012 (D' = 0.39) and 2017 (D' = 0.2).

Moreover, the Gini-Simpson Index, $\bar{\mathrm{D}}$, is following similar annual patterns with the affront mentioned indices (Fig. 5). The value of this index ranges between 0 (all species are equally present) and 1 (one species dominates the community completely). This is because the index gives more weight to the species which is more abundant in the sample and penalizes the addition of rare species because they cause only small changes in its value. In particular, the Gini-Simpson Index $\bar{\mathrm{D}}$ has had its highest value in 2014 ($\bar{\mathrm{D}}$ = 0.93) and 2016 ($\bar{\mathrm{D}}$ = 0.92) and the lowest in 2012 ($\bar{\mathrm{D}}$ = 0.61) and 2017($\bar{\mathrm{D}}$ = 0.8). Therefore, we conclude that during 2014 and 2017 some bird species dominate more compared to the other observation years.

Finally, the Berger-Parker index, D_D, which represents the maximum proportion of any species estimated in the sample assemblage, had its highest values in 2012 (D_D = 0.58) and 2017 (D_D = 0.39) and its lowest in 2014 (D_D = 0.13) and 2016 (D_D = 0.15) (Fig. 5). From a practical standpoint and according to Hubbell's neutral theory (Hubbell, 2001), alterations in the D_D values represent dif-
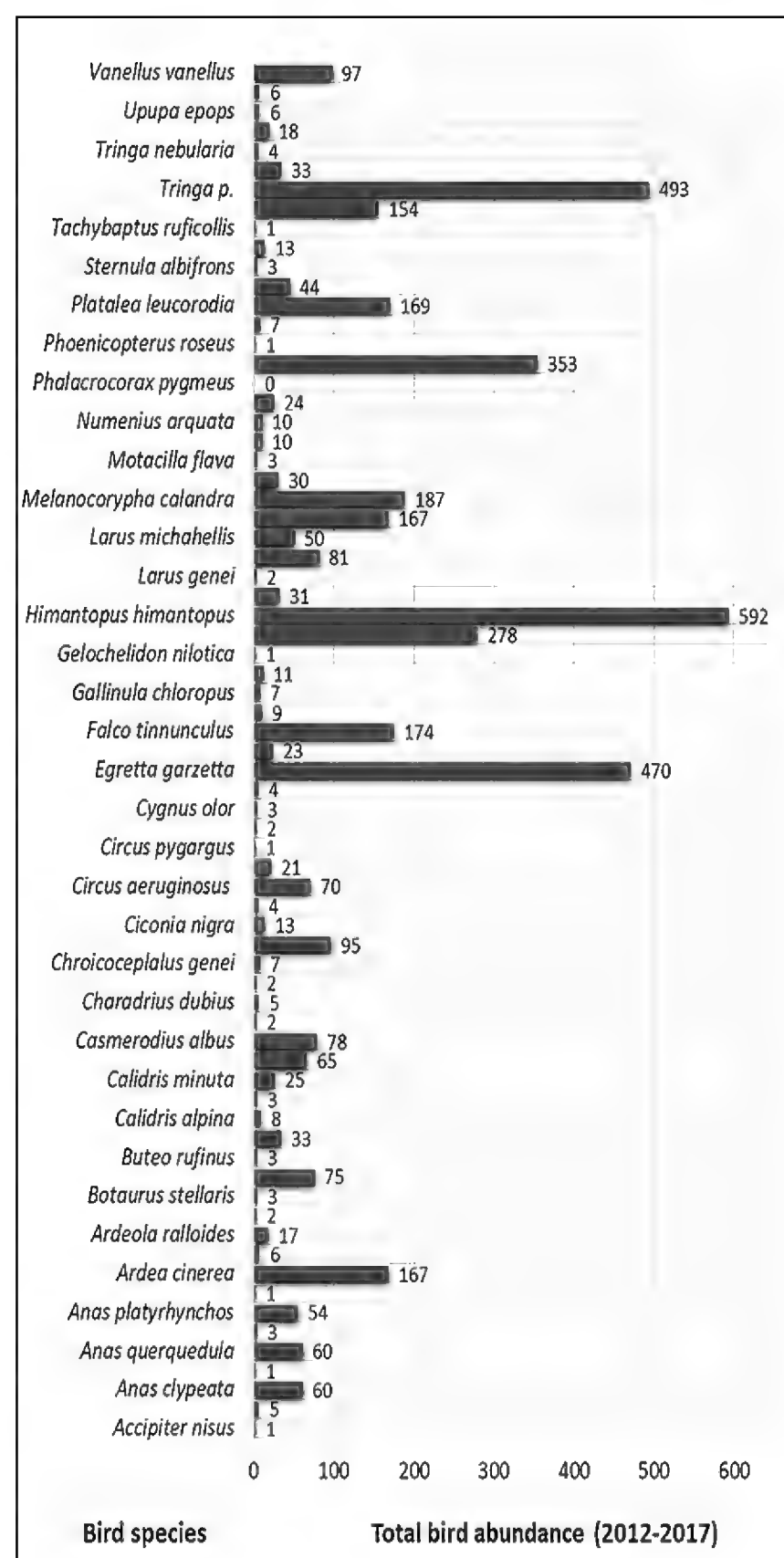
Figure 3. Total bird species abundance recorded inside the spatial protection zone (GR1220010) of the Natura 2000 natural park on the west coast of the Thermaic gulf in Northern Greece from 2012 until 2017.

ferent colonization rates shift distributions towards even patterns which are represented by low D_D values (Caruso et al., 2007). Therefore, successful colonizing bird species have probably dominated more the assemblage in 2017 and 2014 and thus determined the increase in the Berger-Parker index.

Hill numbers biodiversity profiles

A complete characterization of diversity of the

yearly bird assemblages in the Theramaikos gulf Natura 2000 protected area is shown in figure 6 which shows the empirical values of the Hill numbers and the Rényi entropy parameterized by the order q in terms of an empirical curve (McGuiness, 1984).

In particular, the diversity and entropy profiles in figure 7 are plotted for all values from $q = 0$ through infinity, although are not shown from $q = 4$ and beyond, because they do not change essentially and thus do not contain much information on the compositional complexity of the bird assemblages.

The first three numbers coincides with three diversity indices, namely the true diversity of all species ($q = 0$; species richness), the diversity of typical (common) community species ($q = 1$; exponential of the Shannon-Wiener diversity index) and the diversity of the dominant species ($q = 2$; the reciprocal of Simson's diversity). The bars thus represent species and, as the number increases, less weight is given in rare species units of the number decreases and are helpful to assess and compare the yearly time effects on species dominance. In general the profiles of the yearly assemblages do not cross unambiguously between them but for 2015 and 2016, suggesting that these two year were more diverse compared to the other. In most cases the bird assemblages showed similar diversity patterns in respect to the first Hill numbers although the values were different.

The species richness (q = 0) were higher in 2016 ($D_S = 38$) and 2015 ($D_S = 37$), followed by 2014 ($D_S = 31$) and by 2013 and 2017 which have the same levels ($D_S = 29$). The lowest species richness was estimated in 2012 ($D_S = 26$). Moreover, the bird biodiversity (Hill number $q = 1$) were higher for 2014 ($^1D_y = 18.662$) and 2016 ($^1D_y = 17.953$) compared to 2013, 2015 and 2017 which were lower ($9.465 < {}^1D_y < 14.429$). The lowest biodiversity was estimated in 2012 ($^1D_y = 4.521$).

Moreover, the effective number of the dominant bird species (Hill number $q = 2$) were higher in 2014 ($^2D_y = 14.399$) and 2016 ($^2D_y = 12.505$) compared to 2013 ($^2D_y = 10.213$) and 2015 ($^2D_y = 8.629$). The lowest values were estimated in 2017 ($^2D_y = 4.993$) and 2012 ($^2D_y = 2.563$). The above values have biological sense since realistically represent the effective number of bird species (i.e., with the same abundance) in respect to each observation year that theoretically can coexist with the maximum evenness. The Hill numbers q>3 have shown similar patterns with the previous parameters although taking slight lower values, although during 2012 the Hill's curve is more uneven (steeper decline) compared to all other years. Figure 5, shows the pooled over the years values of the Hill number biodiversity indices. The mean species abundance (q = 0) was 31 species, the mean biodiversity (q = 1) was 13 species and the most dominant species (q = 2) were 8 species.

Rényi entropy biodiversity profiles

The Rényi entropy profiles generally follow the same patterns as the Hill numbers in respect to each observation year (Fig. 8). Moreover, in all cases the
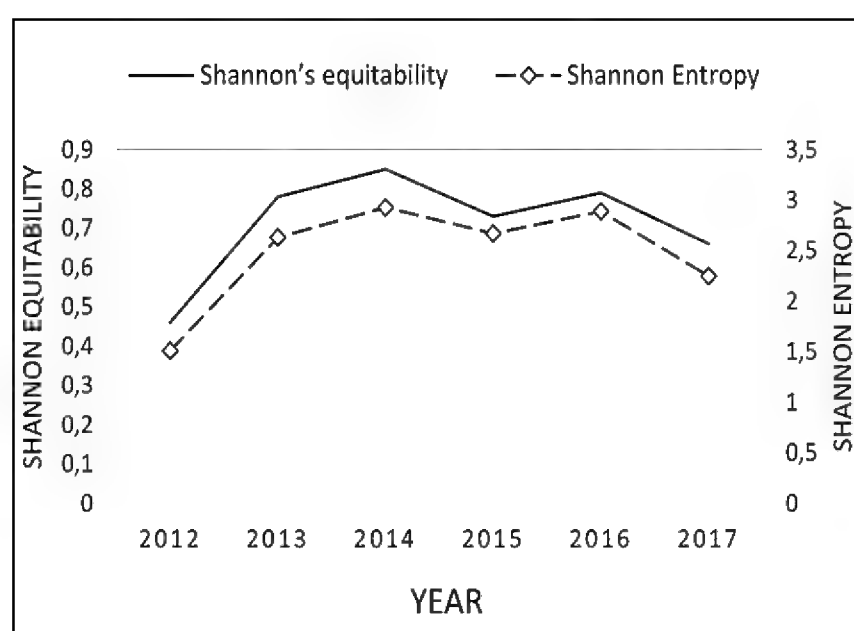
Figure 4. Yearly trends and relative analogies of the Shannon's entropy and related Shannon's equitability index of bird biodiversity of a representative site of the Natura 2000 Thermaikos gulf protected area in Northern Greece.
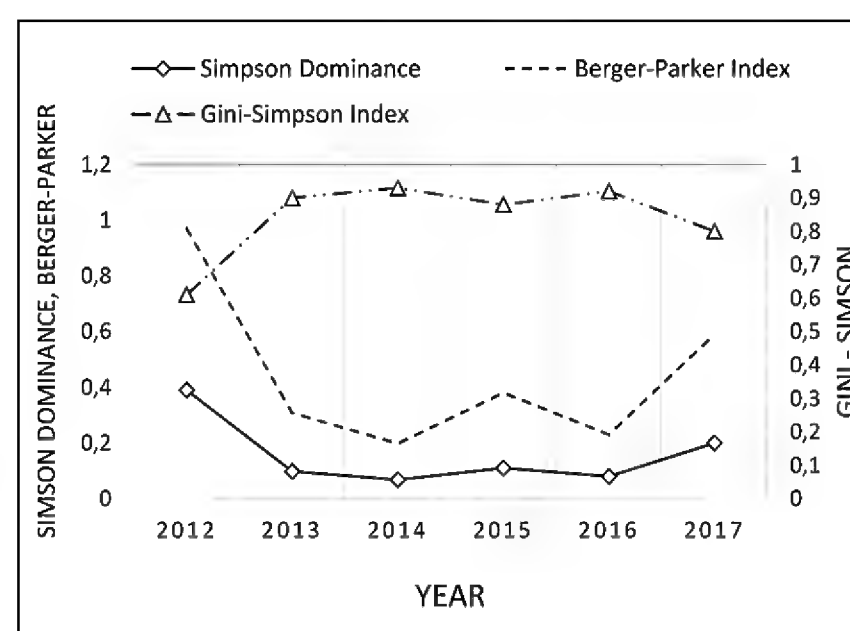
Figure 5. Yearly trends of the Simpson dominance, Gini-Simpson and Berger-Parker indices of bird biodiversity of a representative site of the Natura 2000 Thermaikos gulf protected area in Northern Greece.

Rényi entropy profiles showed analogous diversity patterns as for the first Hill numbers. Higher values were estimated for 2014 and 2015 compared to all other years. This suggest that these two years were more diverse compared to the other. Finally, a slight crossing appears in 2014 and 2016 with 2013 and 2015 when moving from the parameter $q = 0$ towards $q = 1$.

DISCUSSION

Birds are relatively easy to see and count and, due to their sensitivity in habitat conditions, birds can be used as reliable indicators of species biodiversity on practical and scientific grounds. Birds, particularly, are diverse, are high in the food chain, which makes them sensitive to changes at lower food change levels (Thomas et al., 2004), they are also sensitive to landscape modification as well as to the presence of persistent pollutants (Barker & Tingey, 1992; Backhaus et al., 2012). As a result, the quantification of yearly changes in the complexity of bird biodiversity provides important information which potentially can be further used to identify any driving forces behinds their temporal alterations (Crick, 2004; Visser et al., 2006).

Nevertheless, one disadvantage of using birds to estimate biodiversity, in general, as well as in the particular Natura site of interest, is that they do not often reflect of all other taxa in their domain (Gregory, 2006). Moreover, in some cases there are bird species in which it is realy hard to link their population abundances to specific drivers on the ground, since some species may counter positively to ground change, while other negatively. For example the excess of nutrients due to eutrophication may be detrimental in the round of a wetland for some species, but beneficial for others (i.e., wildfowls *Anas platyrhynchos*).

In this work we estimate and present for the first time the compositional diversity of bird abundances at the species level from 2012 to 2017 in one of the most important wetland Natura site in Northern Greece using several biodiversity indices. More than 50 bird species have been listed in total with a combination of water birds, endemic as well as migratory species. The highest values of most biodiversity indices were recorded in 2014 and 2016 suggesting that there is a higher compositional diversity compared to all other years of observation.

The bird biodiversity has been known to play an important role as biodiversity indicator in the well-being and health of ecosystems (Tucker & Evans, 1997). Therefore, there have been important endeavors to describe bird compositional complexity and its stability contributing to the evaluation of the sustainability of specific habitats and ecosystems.

In this work we have addressed the estimation of temporal changes of bird assemblages by means of a series different biodiversity measures, to aid in better understanding of the functioning and sustainability of this important Natura 2000 protected area in Northern Greece.
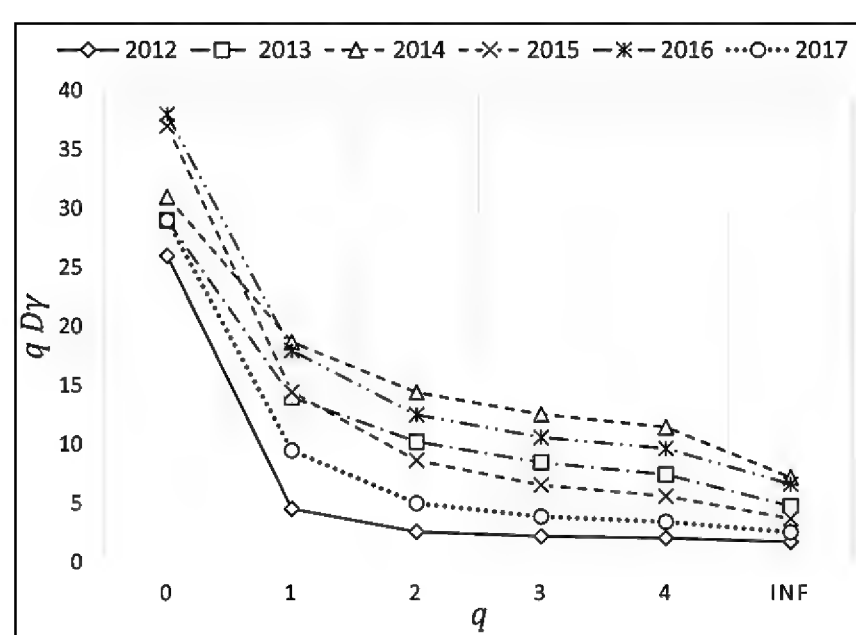
Figure 6. Characterization of the species diversity of an assemblage with Hill numbers that report the diversity of all species (q=0), the diversity of a "typical" species (q=1), and that of dominant species (q=2) as well as following dimensions.
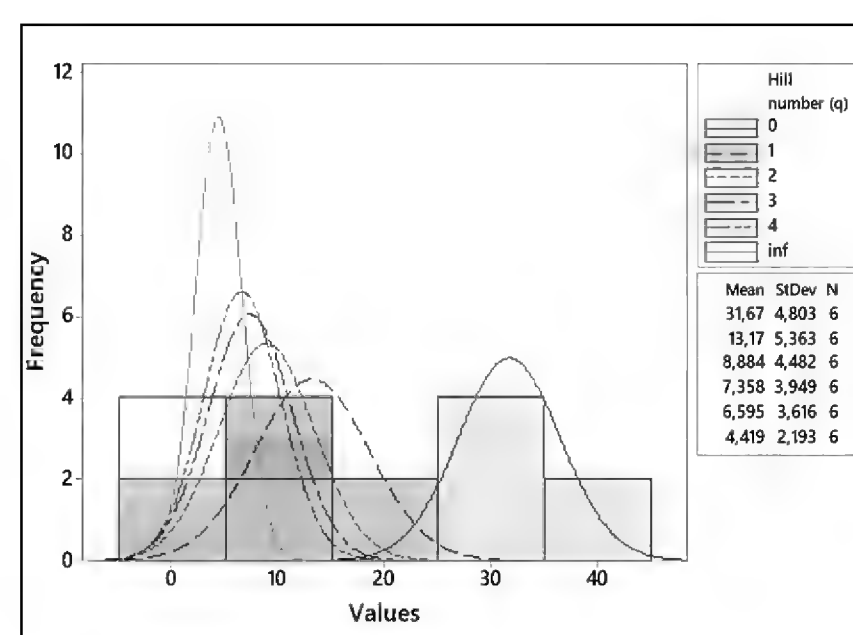
Figure 7. Pooled distribution patterns, first and second moments of the Hill number in estimating the mean bird biodiversity of a representative site of the Natura 2000 Thermaikos gulf protected area in Northern Greece.
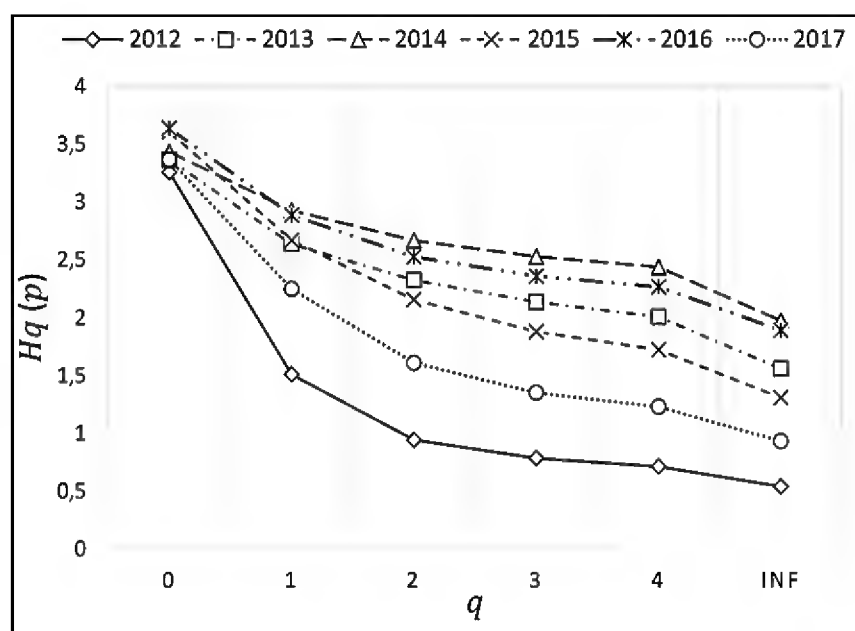
Figure 8. Complete characterization of the species diversity of an assemblage using Rényi entropy. The profile represents the diversity of all species (q=0), the diversity of a "typical" species (q=1), and that of dominant species (q=2) as well as following dimensions (details in text).

Because the concept of biodiversity is rather straightforward considering the partitioning diversity into different components (i.e., richness, evenness, relative abundance etc) (Wagner et al., 2018), we have decided to estimate and compare a series of different biodiversity indices to provide an holistic approach to characterize the particular composition of bird assemblages. Furthermore, we have decided to calculate and compare the time related biodiversity curves for each index to detect alterations and analogies between the different measures. This provide a robust measure of bird compositional biodiversity as well as information upon the changes throughout time.

Although both Shannon's diversity as well as Simpson's index are both estimators of biodiversity, the first put more weight on species richness, while the second, on species evenness (Kim et al., 2017). To date, species richness is the number of different species present in a certain niche which does not take into account the number of each species present in contrast to evenness, which includes information on the uniformity of the population size of each of the species present. Therefore, a major advantage over simple diversity measures, such as richness and evenness, is the fact that they provide more inference about the structural composition of the bird populations. Moreover, although in Shannon's diversity as well as Simpson's index the sample size is generally negligible for both of them, it is often difficult to com-

pare communities that differ greatly in richness based solely on Shannon's diversity.

In the current study the Shannon Entropy had the highest values in 2014 (D_H = 2.927) in 2014 and in 2016 (D_H = 2.888), suggesting that there is a higher diversity compared to all other observation years. These high values may be related to a higher number of species and/or a distribution which is more even compared to other cases (Magurran, 2004). In general, typical values of Shannon Entropy are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4. However, because these values are related to the particular study region, it is difficult to be compared to other regions *per se*. On the other hand, because the Simpson index varies for 0 to 1, it can be probably used to compare more easily relative analogies to species bio diversities of other regions.

Moreover, the empirical curve profiles of the Hill's numbers, as well as the Rényi entropy, provide a quantification in the change in the shapes of the curves over time and the ability to compare their different patterns in relation to the different observation years. In 2012 and 2017, for instance, the shapes of the diversity curves differ considerable compared to all other years and which explains visually the discrepancies in comparing the other diversity indices. In other words, although the other biodiversity indices used evaluated the temporal changes in bird biodiversity, Hill's and Rényi complexity measures may provide different answers related to weight given to rare species (Wagner et al., 2018). Thus, the biodiversity curve profiles have the advantage of providing information about the change in the evenness of the bird assemblages over time in a single model.

However, it is still important to generalize the information from the biodiversity indicators in order to establish a link to the driving causes which explain any ecological variation in bird abundance from year to year as well as from location to location. For instance, when an ecosystem degrade, a few generalist species, that can thrive in wide variety in habitat modified by human, take over a large number of specialist species which are decently strongly by absolute precise habitat conditions. As a result, small population of native species become extinct and a few generalist species dominate. This process of such a temporal change is known as *biotic homogenization* (Thomas et al., 2004). There-

fore, very often neither the rarest nor the commonest species *per se* are reliable indicators of biodiversity. Additionally, alterations of abiotic factors such as changing environmental temperatures, alterations in rainfall regime such as the amount of precipitation (rain, snow, etc.), the relative humidity of the atmosphere, as well as the increasingly frequent extreme weather events are characteristic elements of climate change and can possible affect species phenology, reproduction and biodiversity (Crick, 2004; Visser et al., 2006; Cormont et al., 2011; McMahon et al., 2011; Auer & Martin, 2013).

To conclude, based on the results there were alterations in bird assemblages throughout the observation years and especially during 2012 and 2017. Additionally, because the biodiversity indexes used in this study are representative and sensitive to environmental change they provide a capable quantitative measurement of the species occurred in the particular location of interest and their stability throughout time. Therefore, more work has been planned to answer the question of whether annual changes in the composition bird biodiversity are related to exogenous factors such as climate and/or any human activities. Yet, this study has shown that use of diversity indexes are robust estimators of biodiversity since they give as biodiversity by proxy and save us time and reduce sampling efforts since it is virtual impossible to survey everything. Information from this work should facilitate evidence-based strategies for the preservation and sustainable management of the Thermaicos semi-Agricultural Natura 2000 area of conservation in Northern Greece.

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Growth and length-weight relationships of *Gambusia affinis* (Baird et Girard, 1853) population in Algeria (Cyprinodontiformes Poeciliidae)

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ABSTRACT   The aim of this study is to provide necessary information on the growth, length-weight relationships of *Gambusia affinis* (Baird et Girard, 1853) (Cyprinodontiformes Poeciliidae) in four different areas: Timimoune's foggara, southwestern Algerian Sahara, with a total of 402 specimens; 182 fishes in Bechar; Biskra, northeastern Sahara, with 282 specimens; and Oued Sebaine, in western Algeria, with 638 fishes. The female to male sex ratio was 2.14:1 (Timimoune), 2.19:1 (Bechar), 2.61:1 (Biskra) and 2.35:1 (Tiaret). The length-weight relationship of *G. affinis* population shows a difference in growth between the sexes (isometric, positive and negative allometric). The parameter of Von Bertalanffy, L∞, was obtained for the females of each region: in Timimoune, 62.14 mm; in Bechar, 51.72 mm; in Biskra, 67.25 mm; and in Tiaret, 63.62 mm. The L∞ of males for each region is instead: in Timimoune, 45.48 mm; in Bechar, 41.75 mm; in Biskra, 47.89 mm; and in Tiaret, 45.49 mm.

INTRODUCTION

The birth rate, death rate, population size, density, age structure and sex ratio of population are parameters particularly important that influence population growth or decline (Dodge, 2006). The allocation of energy to growth and reproduction, in relation to temperature and food availability. Vondracek et al. (1988), investigated in laboratory experiments with *Gambusia affinis* (Baird et Girard, 1853). The growth rates of this specie in a cycling temperature regime (20-30°C), were faster than in a 25°C constant temperature, and a negative relationship between growth rate and fish density (Stearns & Sage, 1980).

Gambusia affinis is the most abundant, widespread freshwater fish in the world (Pyke, 2008), it belongs to Poeciliidae family and Cyprinodontiformes order. Originally, it comes from the eastern and south-eastern United State of America, ranging from New Jersey to central Mexico, it has, however, been successfully introduced to most of the world, except Antarctica (Pyke, 2008). In Algeria, *G. affinis* is considered as a species exotic, it was mentioned in the works of zoologist Seurat, declaring the introduction of this species in Algeria since 1924 (Arab, 1989). This species is considered as a biological control agent to eradicate mosquitoes and the disease vectors they harbor (Green & Imber,

1977; Nelson & Keenan, 1992; Offill & Walton, 1999), it is therefore successful in controlling malaria (Krumholz, 1948; Walton, 2007).

This species is a hardy fish which is tolerant to a wide range of water quality parameters, including elevated salinity (Alcaraz & García Berthou, 2007). *Gambusia affinis* from Texas grew more slowly in fresh water rather than in brackish water (Stearns & Sage, 1980), and survives in waters with very low oxygen (Cech et al., 1985), and high temperatures (Pyke, 2005)

The overall aim of this study is to provide original information for the first time on some parameters, such as the length population structure or frequency distribution, length-weight relationship, age and the growth parameters of Von Bertalanffy of the *G. affinis* population in four Algerian biotopes, such as the west, the northeast and the southwest of the Algerian Sahara and compare these results with other studies on mosquitofish. Note that knowledge of these biological characteristics and the length-weight relationships is very important for the assessment and management of fish stocks (Cai et al., 2019).

MATERIAL AND METHODS

Study area

All specimens were collected from four different areas (Fig. 1).

Figure 1. Geographical location of the study area.

Gazelle fountain dam in Biskra (35° 7'25.52"N and 5°35'25.04"E). It is located at 37 km from governorate Biskra (the Ziban), in the northeastern of the Algerian Sahara, at the southern foothills of the Saharan Atlas. The mountains at the northern limit mitigate the extension of the influences of the humid Mediterranean climate and give the region a hyper arid character towards the Sahara to the south. The data show that the coldest month is December with an average temperature of 12.2 °C, and the hottest month is August with 34.7 °C.

Oued Sebaine in Tiaret (35°26'18.01"N and 1°36'31.09"E). It is located in north-western of Algeria, 28 km the east of the governorate Tiaret, at an altitude of 918 m, it is small river that has a ten of permanent sources of fresh water, s'called Ras El Ain, about 1.4 km of long, it poured into the Ouassel river. The continental climate has two periods: a harsh winter with an average temperature of 6 °C in January and a hot and dry summer at 34.9 °C in July. This wadi is a habitat of high terrestrial and aquatic biodiversity.

The Timimoune's foggarain Adrar (29°15'13. 58"N and 0°12'35.19"E). Situated in the western Algerian Sahara, Igraven and Abad are the sampling sites of fish in Timimoune about 200 km northeast of Adrar governate, at 257 m altitude. The foggara is a draining underground tunnel dug in a straight line from upstream to downstream, which collects and brings underground water to the land to be irrigated. The Saharan climate is characterized by a very irregular and weak rain, with an average temperature of 13.2°C in January, and 37.5°C in July.

The small dam in Abadla, Bechar (31°01'13"N and 02°43'14"W). It is located in the southwest of Algeria, at 950 km from the capital Algiers, the small dam is a part of the Oued Guir which originates in the High Atlas in Morocco and travels to the border between Algeria and Morocco, feeds the Djorf Torba dam (50 km west of Bechar), then crosses the town of Abadla. The Saharan climate is characterized by a very hot summer and a very cold winter, light precipitation, frequent and violent winds.

Sampling

Monthly samples were collected for each area, a total of 282 specimens of *G. affinis* during the period from November 2006 to May 2007 at the Gazelle fountain dam in Biskra, 402 specimens

were collected from July 2010 to June 2011 in Timimoune's foggara, 638 fish were collected in wadi Sebaine from March 2017 to February 2018, and 182 mosquitofish were collected between May 2018 and October 2018 in the small dam in Abadla, Bechar, where the sampling stopped due to a flood. All the sampled fish were caught during the day using a dip net (a stretched size of 1 mm). The biological material was preserved in a 4% formalin solution and sent to the laboratory, where the total length (Lt) was measured by a digital calliper (to the nearest 0.01 millimeters) and the weight by a digital precision balance (to the nearest 0.01 grams). The sex was determined from the morphology of the anal fin, which takes a gonopodium form, and by direct observation of the gonad structure.

The test of reduced gap ε used for the comparison of the average sizes of males and females (Schwartz, 1983) followed this equation:

$$\varepsilon = \frac{|\overline{X_1} - \overline{X_2}|}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

Where σ_1^2: variance of females; σ_2^2: variance of males; X_1: denotes mean female; X_2: denotes mean male; n_1: number of females; and n_2: number of males.

Length-weight relationship

The length-weight relationship was calculated for both sexes using the allometric equation (Le Cren, 1951): $Wt = a \times Lt^b$.

Where Wt is the body weight (g), Lt is the total length (mm), a is the regression and b is the regression coefficient (slope),. The parameters a and b were estimated by linear regression analysis by logarithmic transformed (Froese, 2006) as follows: $Log(Wt) = Log(a) + b \times Log(Lt)$.

If b = 3 the growth is isometric, if b < 3 the allometry is negative and if b > 3 the allometry is positive.

Growth parameters

The growth model used for the fish growth is that of Von Bertalanffy (1938), according to the following equation : $Lt = L_\infty (1 - e^{-K(t-t0)})$.

Where L_∞ is the asymptotic total length, Lt is the total length in mm at age t, K is the growth coefficient and t0 is the hypothetical age at which length is zero.

Statistical processing

The growth parameters of the Von Bertalanffy equation were estimated by the ELEFFAN method (Electronic length-frequency analysis) of the LFDA software (length frequency distribution analysis) (Kirkwood et al., 2001).

The growth performance index Φ'proposed by Pauly and Munro (1984), was calculated using the following formula: $\Phi' = log(K) + 2 \times log(L\infty)$.

RESULTS

Population structure and Length frequency distribution

The population of *G. affinis* presents in Biskra consists of 282 individuals, 204 females (72.34%) and 78 males (27.65%), in Timimoune 402 fish were captured, 274 females (63.11%) and 128 males (36.88%), in Tiaret 638 mosquitofish were captured, 448 females (70.21%) and 190 males (36.88%), and in Bechar 182 fish were captured, 125 females (68.68%), and 57 males (31.31%). Therefore, the overall sex ratio (F:M) of *G. affinis* for each area is in favor of females rather than males: Biskra, 2.61:1; Timimoune, 2.14:1; Tiaret, 2.35:1; and Bechar, 2.19:1. These values were significantly different from 1:1.

The length of *G. affinis* females and males in Biskra ranged, respectively, from 19.01 mm to 64.02 mm and from 21.9 mm to 45.07 mm. In Tiaret the minimum and maximum size of females and males ranged, respectively, from 16.6 to 60.20 mm and from 18.7 to 43.07 mm. In the population of Timimoune the total length of the females and males were, respectively, from 19.01 mm to 59 mm and from 21 mm to 43 mm. Lastly, the length of the population of females and males in Bechar ranged, respectively, from 16.7 mm to 49.10 mm and from 19.73 mm to 38.05 mm.

The calculated values of the reduced gap ε for our study are 3.62 (in Biskra), 11.94 (in Timimoune), 4.11 (in Tiaret) and 4.97 (in Bechar). These values are higher than the value given to the Gaussian distribution Table (1.96) for a confidence level of 5% indicating that the average sizes of females is significantly larger than that observed for males.

Figure 2 shows in Timimoune that there are more
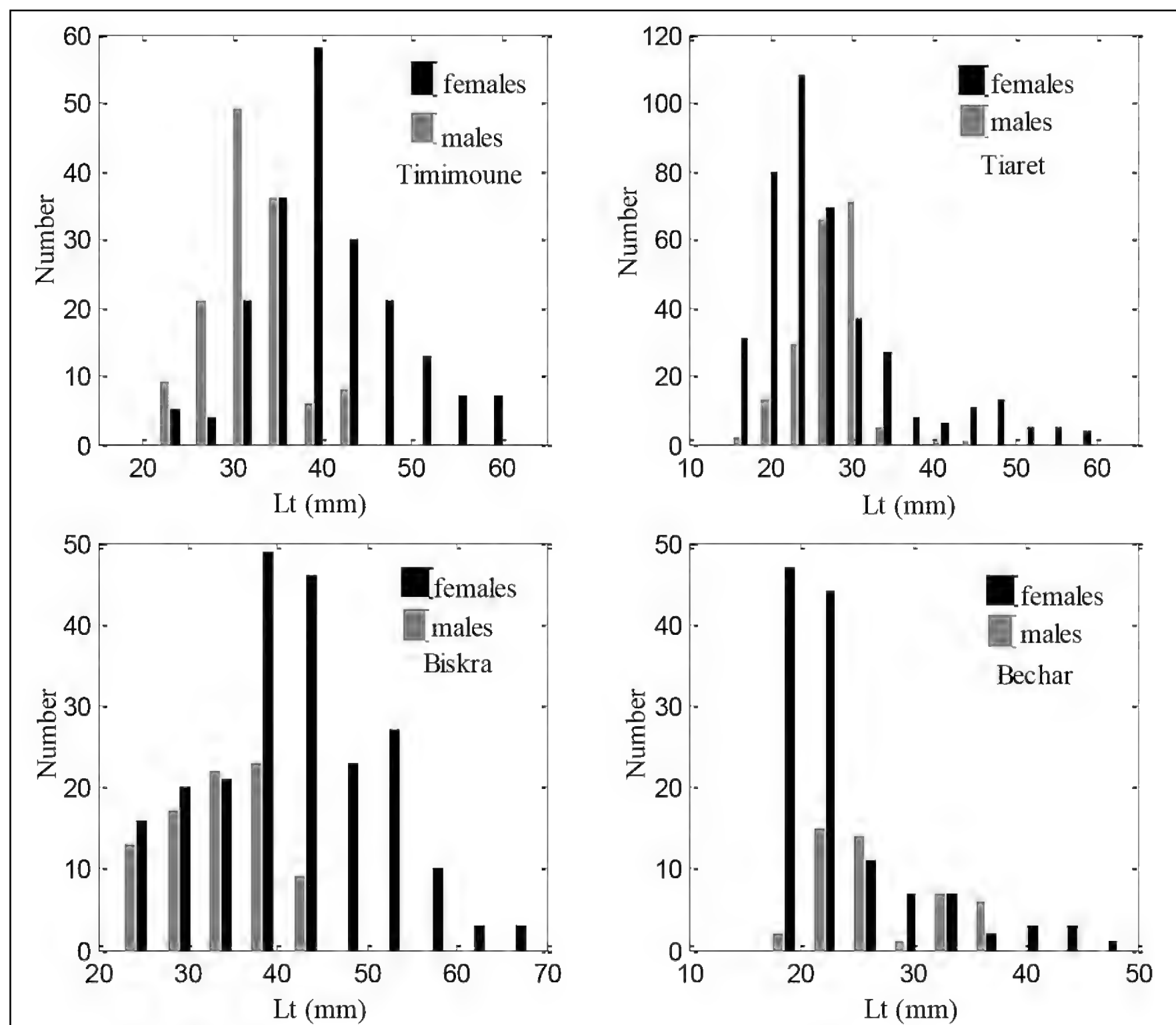
Figure 2. Length composition of *Gambusia affinis* from each region.

females than males in all size groups except 23.01 mm (5 females/9 males), 27.01 mm (4 females/21 males) and 31.01 mm (21 females/49 males). In Tiaret the females were more than males in all size groups except 30.35 mm (37 females/71 males), hence in Biskra and Bechar, females were generally the most abundant in all sizes, while males of Bechar predominated in sizes 25.7 mm (11 females/14 males) and 36.5 mm (2 females/6 males).

Length-weight relationship

Length-weight relationship of *G. affinis* is illustrated in figures 3, 4, 5 and 6 which show a negative allometry growth observed for males of Timimoune (b = 2.82), Tiaret (b = 2.81) and Bechar (b = 2.84),

and a positive allometric for females of Biskra (b = 3.19), Timimoune (b = 3.07) and Tiaret (b = 3.32), and isometric growth for males of Biskra (b = 3.00) and females of Bechar (b = 3.04). All linear regressions of the length-weight relationships were very significant, with the coefficient of determination (r^2) of Biskra, 0.980 for females and 0.928 for males, of Timimoune, 0.897 for females and 0.906 for males, of Tiaret, 0.986 for females and 0.925 for males, and of Bechar, 0.963 for females and 0.952 for males, with (p <0.05).

All available data on the Von Bertalanffy growth equation and Φ' values of the *G. affinis* population from the present study are compiled in Table 1, in which the Φ' index shows a great similarity between the two sexes.
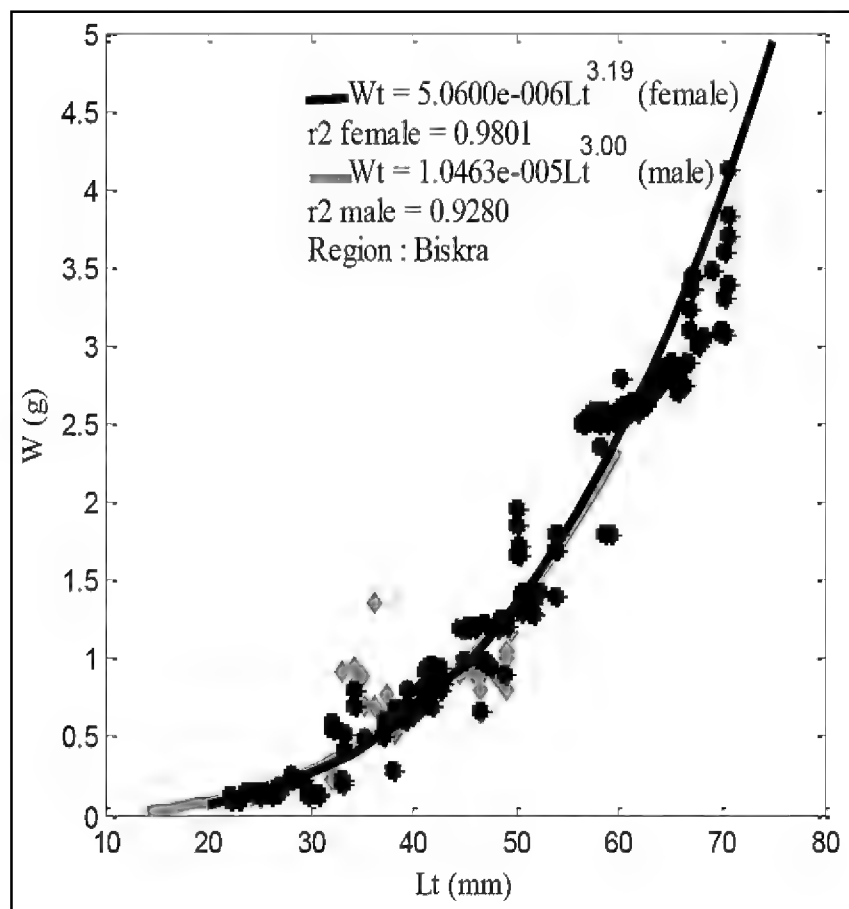
Figure 3. Length-weight relationship of male and female *Gambusia affinis* at Biskra.
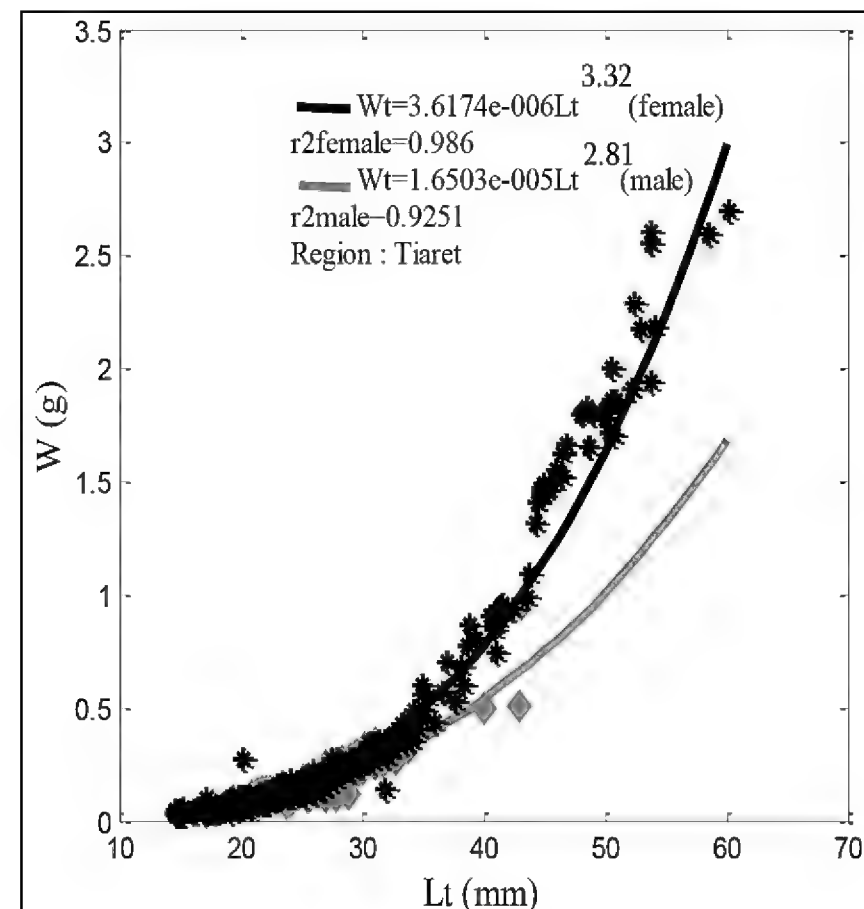
Figure 5. Length-weight relationship of male and female *Gambusia affinis* at Tiaret.
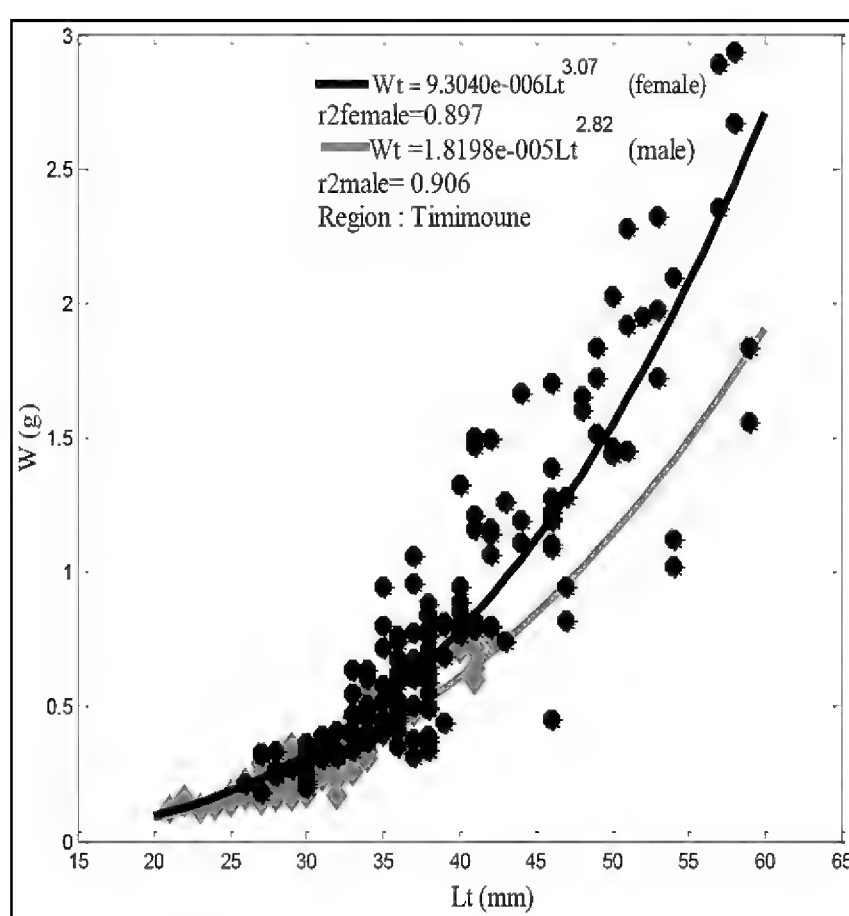
Figure 4. Length-weight relationship of male and female *Gambusia affinis* at Timimoune.
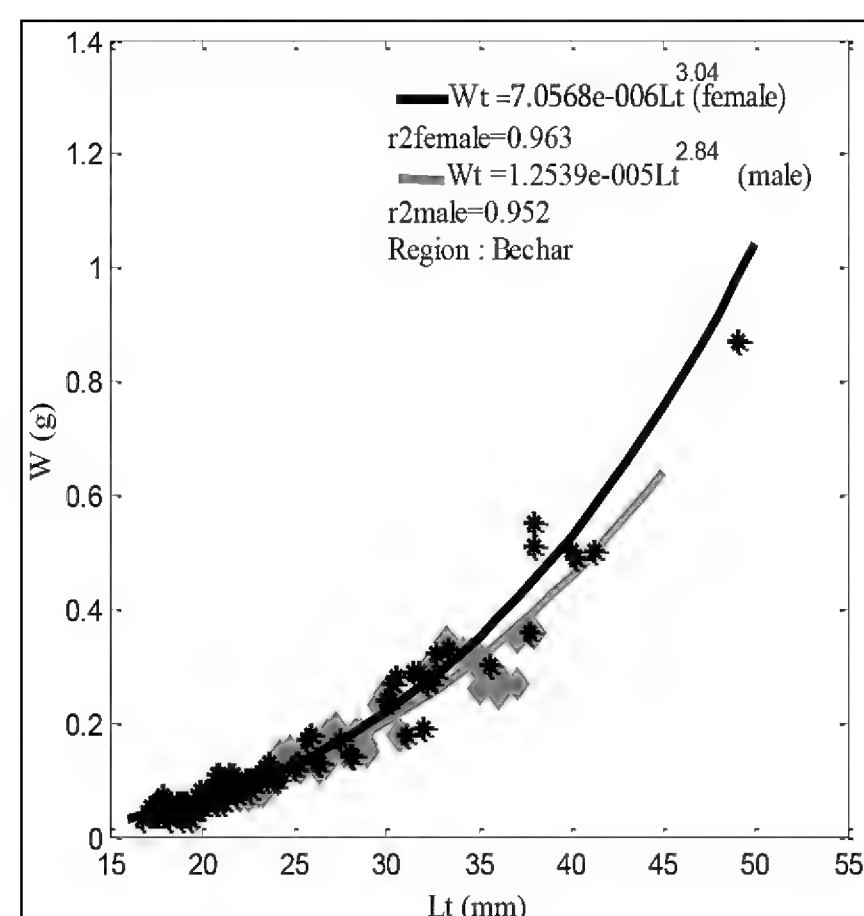
Figure 6. Length-weight relationship of male and female *Gambusia affinis* at Bechar.

DISCUSSION

The overall sex ratio of *G. affinis* (F: M) in the four populations of Biskra, Timimoune, Tiaret and Bechar was significantly different from a 1:1 in favor of females, as confirmed in previous works by Öztürk & İkiz (2004). However, the sex ratio is different at Althama (1:1.1), Aian Elmajdob (1:2.5) and Ard Alahlam (1:2) in Benghazi, Libya (Eltaeeb & El-baraasi, 2019). According to Krumholz (1948), the sex ratio of *Gambusia* at birth is 1:1, but this ratio is altered due to different sampling strategies, and sam-

Areas	Von Bertallanffy Equation				
	Female	Φ'	Male	Φ'
Biskra	$L_t = 67.25\,(1-e^{-0.99\,(t + 0.58)})$	3.65	$L_t = 47.89\,(1-e^{-0.90\,(t + 0.42)})$	3.31
Timimoune	$L_t = 62.14\,(1-e^{-0.89\,(t + 0.99)})$	3.53	$L_t = 45.48\,(1-e^{-0.97(t + 0.95)})$	3.30
Tiaret	$L_t = 63.62\,(1-e^{-0.80\,(t+0.73)})$	3.51	$L_t = 45.49\,(1-e^{-0.99\,(t+0.53)})$	3.31
Bechar	$L_t = 51.72\,(1-e^{-0.72\,(t+0.43)})$	3.28	$L_t = 41.75\,(1-e^{-0.46\,(t+0.13)})$	2.90

Table 1. Von Bertalanffy growth equation of *Gambusia affinis* for both sexes.

Authors	a	b	r2	Sex	Areas
Yu Cheng et al., 2018	$7.13e^{-6}$	3.253	0.961	F	Yangtze River, China
	$3.29e^{-5}$	2.593	0.763	M	
Sibel & Deniz, 2008	0.016	2.626	0.908	F	Lake Adana, Turkey
	0.019	2.474	0.892	M	
Öztürk & İkiz, 2004	0.131	3.230	0.991	F	Fethiye-Akgöl Turkey
	0.135	3.006	0.919	M	
	0.133	3.270	0.988	F+M	
	0.123	3.301	0.987	F	Dalaman, inTurkey
	0.118	2.673	0.812	M	
	0.126	3.27	0.982	F+M	
	0.130	3.3014	0.987	F	Ortaca in Turkey
	0.161	2.6737	0.812	M	
	0.132	3.2685	0.794	F+M	
İlhan & Sarı, 2015	0.0145	2.945	0.818	F+M	Marmara lake, Anatolia, Turkey
Bounaceur, 1997	20.8×10^{-3}	3.20	0.995	F+M	Tonga lake, Algeria
	20.1×10^{-3}	3.17	0.996	F+M	Oubeira lake, Algeria
	32.6×10^{-3}	3.19	0.994	F+M	Oued Bouarroug, Algeria

Table 2. Length-weight relationship of *Gambusia affinis* in different areas of world.

Areas		L∞ (mm)	K (mm / year)	Φ'	Sex	Authors
France	Vistre	78.33	0.25	3.19	♀	Carmona-catot et al., 2014
	Orb	35.81	2.05	3.42	♀	
	Bordigou	43.35	1.00	3.27	♀	
Spain	Fluvia	46.06	1.04	3.34	♀	Carmona-catot et al., 2014
	Ter	31.43	2.79	3.44	♀	
	Ebro	47.43	0.91	3.31	♀	
	Algar	41.71	1.28	3.35	♀	
	Segura	34.67	2.74	3.52	♀	
Hawaii	kay	20.51	1.99	2.92	♂	Stearns, 1983
	twin	60.91	0.53	3.29	♂	

Table 3. Von Bertalanffy growth parameters of *Gambusia affinis* reported in several areas.

pling errors, preferences and changes in their microhabitat preferences at specific stages of life of species between sex (Fernández-Delgado & Rossomanno, 1997). The predominance of females is also due to the longer lifespan (Krumholz, 1948; Kartes & Quignard, 1984; Fernández-Delgado, 1989).

We also observed that the proportion of females increased in the large length classes, and males in the smallest length classes, as females take longer to mature and continue to grow throughout their life (Vargas & Sostoa, 1996), whereas males stop growing or grow very soon after the formation of the gonopodium (Krumholz, 1948).

The length-weight relationships obtained in our regions are strongly correlated (r^2), and suggest differences in growth between the sexes. The females of Biskra, Timimoune and Tiaret exhibit significant positive allometric relationship, indicating that the body weight grows slightly faster than the body length (Froese, 2006), while the males of Timimoune, Tiaret and Bechar present negative allometric growth indicating that the length increases as the cube of weight. For the males of Biskra and the females of Bechar, the isometric growth denotes as the weight and length of fish increases at the same time. Similar growth was observed for females and males of *G. affinis* (Table 2) in the Yangtze river in China (Yu Cheng et al., 2018), in Dalaman, and Ortaca in the Western Mediterranean region of Turkey (Öztürk & İkiz, 2004). Additionally, in northeastern Algeria, positive growth for both sexes has been noted

(Bounaceur, 1997), while in lake Adana in Turkey (Sibel & Deniz, 2008) and lake Marmara, Anatolia in Turkey (İlhan & Sarı, 2015) negative growth for both sexes has been noted.

The b parameter may vary depending on temperature and seasons, habitat characteristics such as salinity, availability of food resources and gonad maturity (Gonçalves et al., 1997; Taskavak & Bilecenoglu, 2001; Özaydin et al., 2007).

The L∞ values (Table 1) obtained for the females and males of Biskra, Timimoune, Tiaret and Bechar indicate that the females have a higher asymptotic length than the males. The growth parameters (L∞, t0 and k) have been compared with other studies (Table 3), and the asymptotic length (L∞) is thereabouts similar to the studies carried out in the regions of Spain, Fluvia and Ebro areas (Carmona-catot et al., 2014). However, the L∞ values are lower than those reported from Vistre, Orb and Bordigou in France (Carmona-catot et al., 2014), and Ter, Algar, Segura in Spain (Carmona-catot et al., 2014), for L∞ of males, the lowest values in Kay and Twin areas in Hawaii (Stearns, 1983). According to Da Silva et al. (2020), the parameters (L∞ and k) apply to the assessment of growth, dynamic characteristics of populations and biomass. Furthermore, the performance index Φ' (Table 1) shows a strong similarity between the two sexes, as these values are similar to those observed in France and Spain (Carmona-catot et al., 2014) and in Hawaii (Stearns, 1983).

CONCLUSIONS

This study is the first data on the biological characteristics such as length-weight relationships and growth parameters of the exotic fish species *G. affinis* in the western mainland of Algeria (Tiaret) and southwestern (Timimoune and Bechar) and northeastern Sahara (Biskra).

The length-weight relationship of *G. affinis* in these areas shows a difference in growth between the sexes indicating the isometric growth, positive and negative allometric growth. Von Bertalanffy's growth parameters differed significantly between sexes and by regional characteristics.

Growth parameters and the the length-weight relationship remain very important for any estimate of fish biomass.

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Evaluation of a dinoflagellate bloom in a perennial endorheic Mediterranean pond

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ABSTRACT   This study is the first to describe a dense dinoflagellate bloom from an inland water body in the Maltese Islands. The bloom in the perennial pond at L-Għadira ta' Sarraflu, in Gozo, Malta was first noted towards the end of April 2020 and was investigated in May 2020. Microscopic analysis indicated a unialgal bloom comprised predominantly of *Peridinium* cf. *cinctum* (O.F. Müller) Ehrenberg (Dinoflagellata Dinophyceae Peridiniaceae) at a mean density of ca. 358118 ± 165227 cells mL-1. Five water quality parameters (pH, oxidation-reduction potential, dissolved oxygen concentration, electrical conductivity and temperature) were measured to provide a preliminary assessment against which future studies could compare. The absence of any long-term abiotic data from the pool precludes any definite causes of the bloom from being identified and highlights the necessity of such a programme.

KEY WORDS   brackish pond; inland waters; Maltese Islands; *Peridinium*.

INTRODUCTION

Algal blooms, defined as episodes during which the density of phytoplankton increases by several orders of magnitude relative to background levels, are a potential occurrence in enclosed aquatic systems. Although many groups of algae are known to form blooms (Smayda, 1997), some of the best characterised are those formed by dinoflagellates (Chromista Dinophyceae). The potential causes of blooms are multiple and probably interactional, and include nutrient spikes, thermal instability, reduced water movement and ecosystem imbalance (Hughes et al., 1999). Dinoflagellate blooms are frequently the basis of a Harmful Algal Bloom (HAB) although this is more often the case in the marine environment as most dinoflagellate blooms in freshwater are benign (Carty & Parrow, 2015). Given that dinoflagellates

are generally situated towards the base of food webs, an understanding of the underlying causal mechanism of a bloom would provide a deeper understanding of the role of bottom-up control in ecosystems in which they occur (Thompson et al., 2008).

The present work describes a dinoflagellate bloom that occurred in the perennial rain-fed pond at L-Għadira ta' Sarraflu, in western Gozo, Maltese Islands. The study was initially commissioned from one of the authors (SL) by the Environment and Resources Authority (ERA) and is now part of a long-term monitoring programme to anticipate such blooms and determine the cause of their formation. To the authors' knowledge, there are no published records of dinoflagellate blooms from inland waters of the Maltese Islands, although this in itself is probably a reflection of the dearth of such wetlands. Unpublished records of dinoflagellate-dominated

blooms in coastal or open marine conditions do exist but are generally descriptions of isolated events without any long-term context (Lanfranco et al., 2018). As such, the present work represents the first published characterisation of a dinoflagellate bloom in an inland wetland in the Maltese Islands.

MATERIAL AND METHODS

Study area

L-Għadira ta' Sarraflu (henceforth referred to as 'SRF') is situated in western Gozo (Fig. 1) and is one of only two perennial lentic wetlands in the Maltese Islands.

The pond margin is approximately circular, with primary and secondary axes measuring 40 m and 34 m respectively, covering an approximate surface area of 1075 m². The maximum water depth reaches ca. 3 m and is limited by the presence of two artificial outflows that drain the pond. In the absence of local geological or hydrological configurations to support the formation of a natural pond of these dimensions, anecdotal evidence suggests that the pond is, at least in part, man-made, and that it has increased in size over the past ca. 50 years.

Field methods

The study was based on a field visit to SRF on Saturday, 9th May 2020, approximately ten days after the discolouration event was first noted. The water body was surveyed to identify any anomalous characteristics relative to those observed during the authors' previous visits to the site. Upon visual inspection, a relatively clumped and static dark-brown discoloration of the water column was immediately evident. Agitation of the water surface by waterfowl led to the emergence of fresh clumps of discoloured water from deeper parts of the water column. The pond and its margins were subsequently visualised from the air using a drone flying at approximately 65 m above the water surface and a photograph encompassing the whole pond was taken (Fig. 2).

Figure 2 was subsequently processed into a pseudocolour image (PCI) using the method described by Bellia & Lanfranco (2019). Image processing was carried out using the k-clustering segmentation algorithm with a k value of 6 in ImageJ v.1.53c (Schneider et al., 2012) to show the spatial extent of the discolouration (Fig. 3).

Five physicochemical properties of pond water
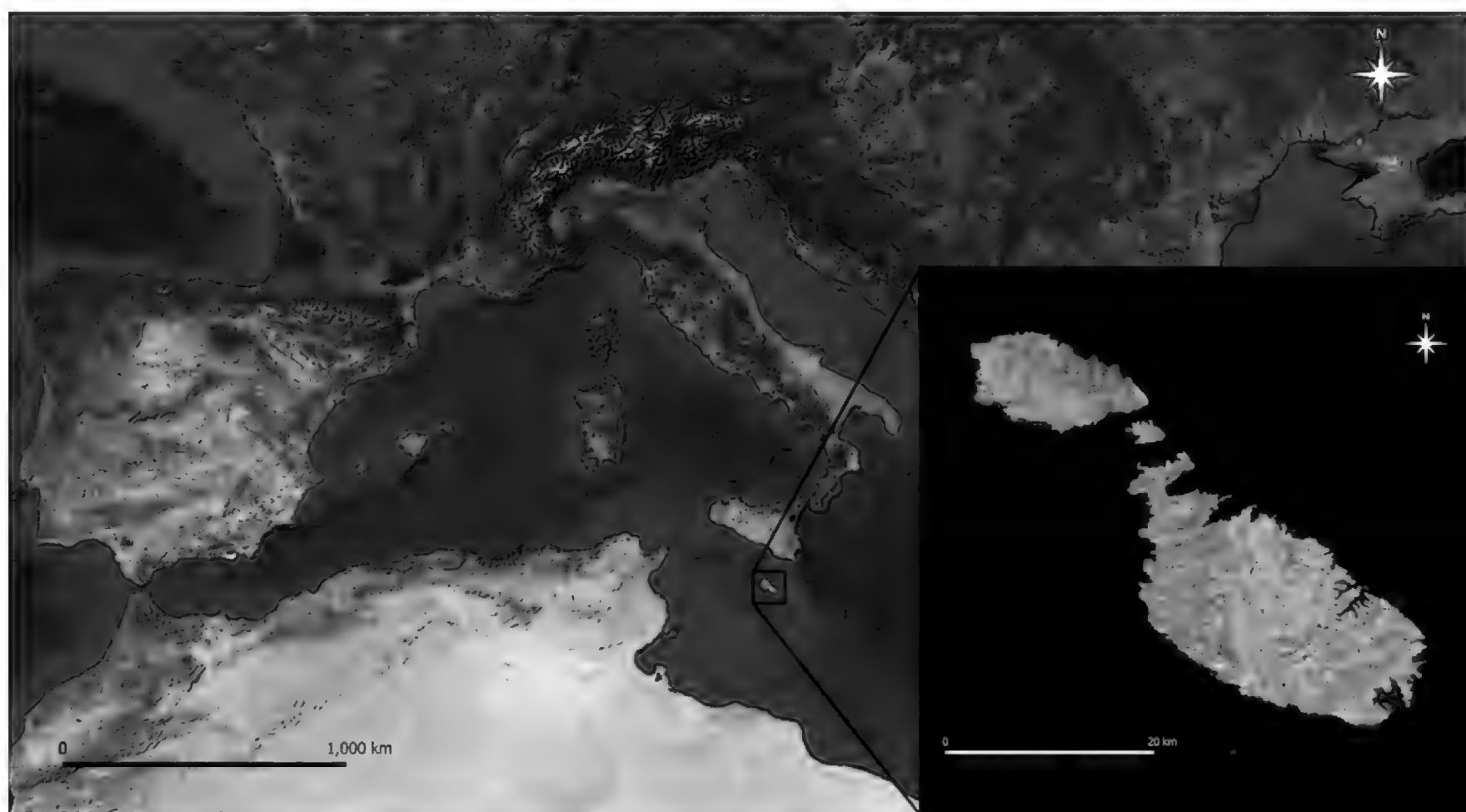
Figure 1. Geographical location of the Maltese Islands in the Mediterranean context, with L-Għadira ta' Sarraflu (SRF) (Latitude: N 36.0366667°, Longitude: E 14.1991667°) indicated by a red dot. Scale bars to the bottom left and North at the top right of each map. Base map obtained from ESRI Satellite (QGIS 3.12.0 'Bucureşti').

were measured *in situ* using a calibrated Hanna Instruments HI98914 multiparameter meter. The properties measured were electrical conductivity (EC) in µS cm^{-1}, *pH*, oxidation-reduction potential (ORP) in mV, water temperature in °C and dissolved oxygen concentration (DO) in mg L^{-1}.

Thirteen independent measurements were taken along the accessible margins of the pond by immersing the probe just below the water surface. The instrument readout was given time to stabilise, after which the measurement was logged automatically.

Results were subsequently compared with archive data from rain-fed water bodies in the Maltese Islands, characterised as 'coastal' or 'inland' according to their geographic location. Archive data were collected by the authors during the same wet season using the same instrument. Replicate water samples were then collected from four accessible sampling points along the pond's margins using a plankton net (mesh size: 10 µm) with a 100 mL sample bottle. The plankton net was 'swept' just below the water surface for a standard length of approximately one metre. Five such sweeps were carried out at each sampling point. The water collected from each set of five sweeps was then transferred to a separate 1L sample bottle for each sampling point. Two more 1L bottles were filled with water through direct immersion. Following collection, the samples were stored in cool, dark conditions and promptly transported to the laboratory for microscopic analysis.

Laboratory methods

Microscopic analysis of the pond water started approximately three hours after collection of the samples to minimise any changes to the phytoplankton content of the samples. Phytoplankton were identified on the basis of their morphological characteristics, dimensions, and patterns of movement. The principal sources used for identification were Carty (2014), Bellinger & Sigee (2015), Carty & Parrow (2015), Moestrup & Calado (2018), and Kretschmann (2020).

The first stage of analysis involved the microscopic observation of pond water at x100 magnification. This was done to visualise any biota present in the living state, where behavioural patterns could be observed. All six sample bottles were agitated

Figure 2. Aerial photo of L-Ghadira ta' Sarraflu (SRF) taken at 65m above the water body. North is indicated by the black arrow. The scale is in the bottom right-hand corner.
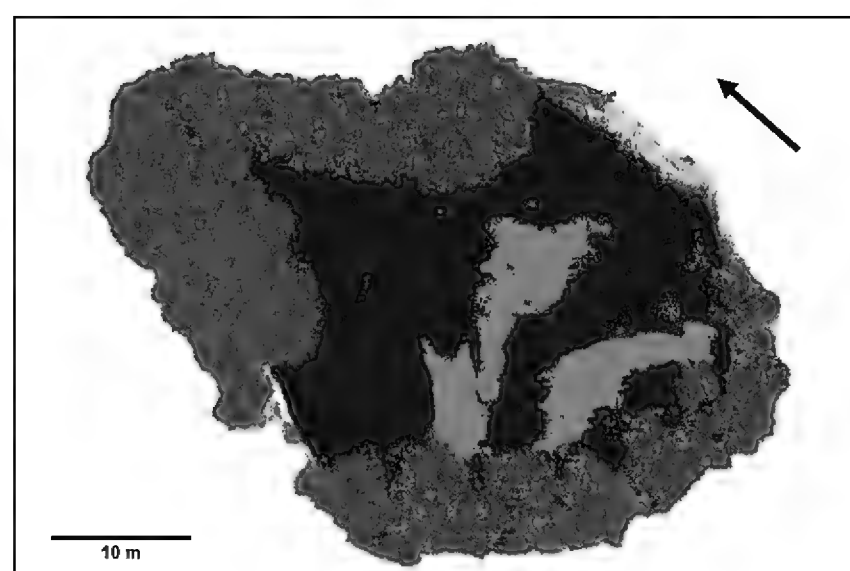
Figure 3. *k*-clustered pseudocolour image (PCI) with *k*=6 of L-Ghadira ta' Sarraflu (SRF) based on an aerial photograph taken from an altitude of 65m above the water body. The orange patches in the water body indicate the extent of the discolouration. North is indicated by the black arrow at the top right, along with the scale at the bottom right.
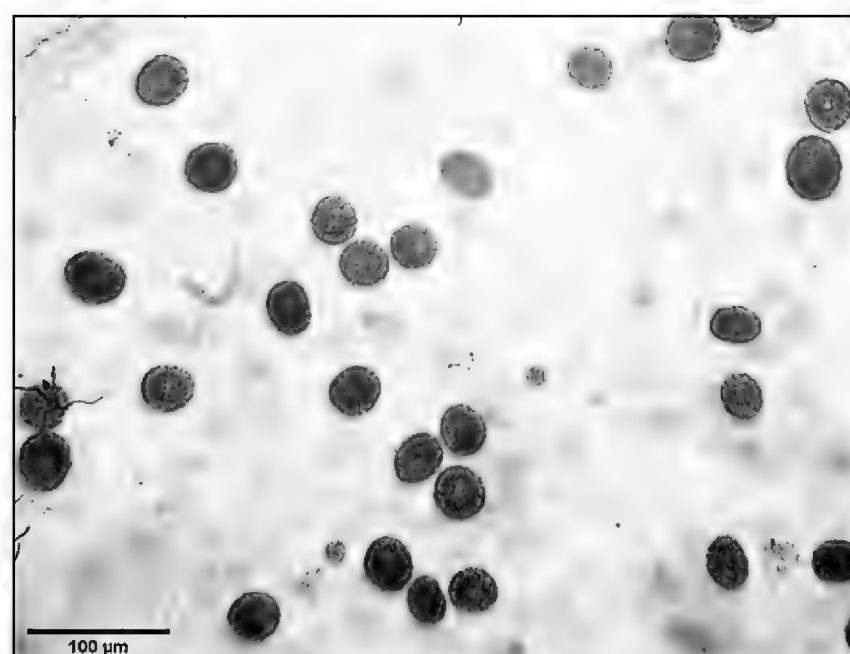
Figure 4. *Peridinium* cells photographed at x250 magnification with visible equatorial furrow in many cells.

vigorously, and a water droplet from each was placed on a microscope slide. The live preparation was observed through an inverted microscope, to which a Swiftcam 10MP camera was attached. No staining or specialised optical procedures were carried out, to minimise disturbance to the organisms present. Flagella were not visualised directly, but their presence was inferred through the cells' patterns of movement. Further analysis of morphological characteristics was carried out at x400 magnification. The water in each of the six sample bottles was then fixed with Lugol's Iodine (Fig. 4), using the procedure recommended by Bellinger & Sigee (2015).

Following fixation, the sample bottles were agitated vigorously, and two 5mL subsamples pipetted out of each and placed in separate Petri dishes. Each Petri dish was observed through an inverted microscope at x100 magnification and 10 micrographs of the visual field were taken from random areas of each Petri dish (Fig. 5). A random sample of 1000 cells from the micrographs were subsequently processed in ImageJ to obtain data for cell dimensions and abundance. Dimensions were characterised by measuring cell diameters using the 'Measure' tool.

Automatic counting of cells was carried out following a 'training' phase, during which the size range and shape characteristics of the cells were determined. The 'training' data was subsequently used to identify and count cells in each image using the 'Analyze Particles' tool (Fig. 6).

Meteorological data

The average air temperature for the period from 1st March, to 31st May were obtained from the Meteorological Office at Malta International Airport, situated approximately 33km southeast of SRF. This was done to identify any trends or spikes in temperature that may have contributed to the bloom's formation.

RESULTS

The anomalous discolouration was mainly concentrated in two high-density plumes, covering approximately a quarter of the water surface at the time of survey. No discernible currents were present beyond surface-water agitation by a light breeze and by waterfowl.

Physicochemical properties of pond water

Figure 7 to Figure 10 compare the four water parameters (EC, pH, ORP and DO) measured at SRF, with the authors' archive data for coastal and inland rainwater pools. This was done to place the pond in a broader context of rain-fed waterbodies situated ca. 30 m from the seashore, and several kilometres from the coast respectively. EC values for SRF ranged from 2615 μS cm^{-1} to 2663 μS cm^{-1} (mean: 2636.7 $\pm$ 14.8μS cm^{-1}) and were found to be much higher than those of the coastal and inland pools

Figure 5. Micrograph at x100 magnification of *Peridinium* cells stained with Lugol's iodine, for processing in ImageJ to obtain cell diameters.
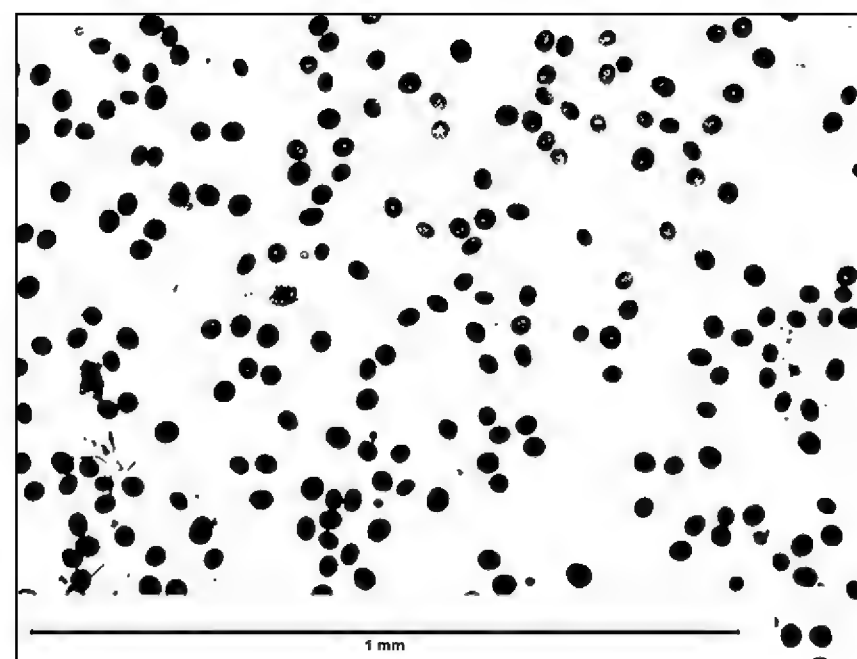
Figure 6. Micrograph at x100 magnification of *Peridinium* cells stained with Lugol's iodine following image processing training phase in ImageJ for cell enumeration.
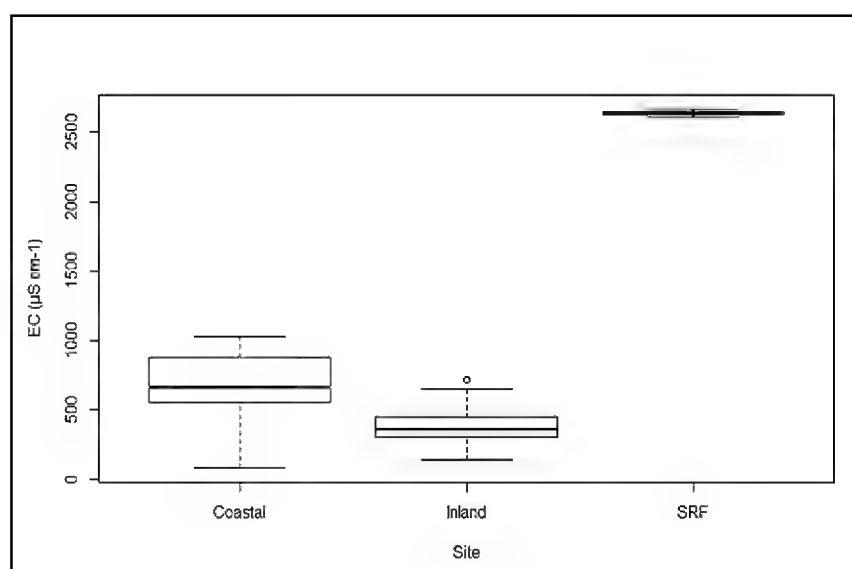
Figure 7. Relative variation of EC of pond water at L-Għadira ta' Sarraflu (SRF) ranging from 2615μS cm⁻¹ to 2663μS cm⁻¹ (mean: 2636.7 ± 14.8μS cm⁻¹), compared with coastal pools (689.7 ± 244.2μS cm⁻¹) and inland pools (383.5 ± 161.6μS cm⁻¹). Comparative data from coastal and inland wetlands collected by the authors during April and May 2020.
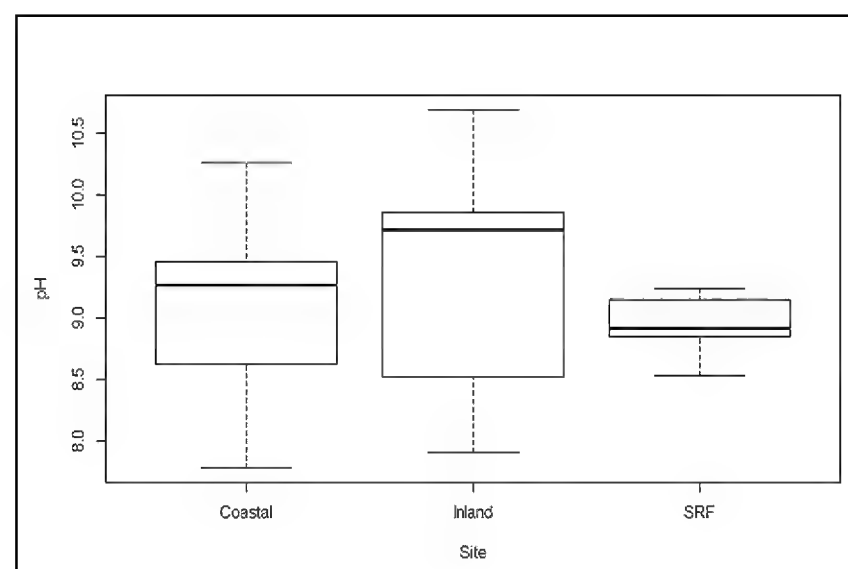
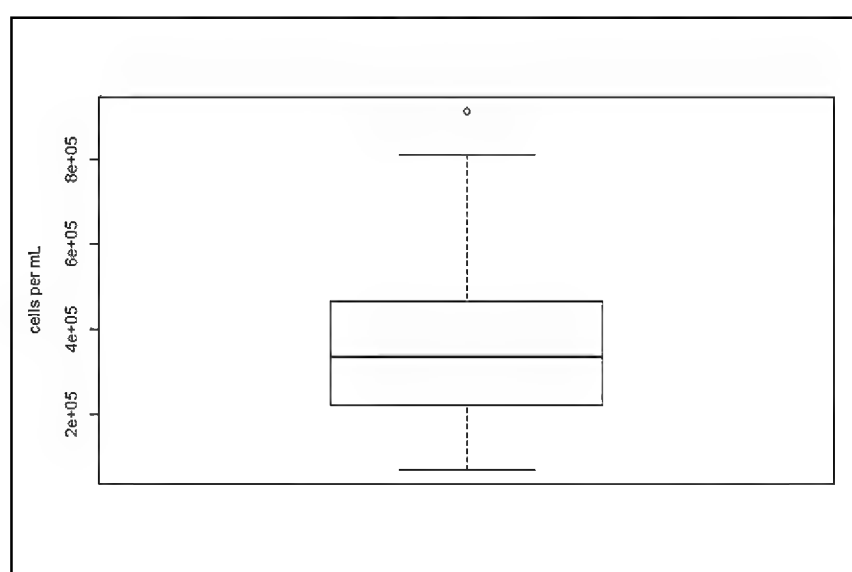Figure 8. Relative variation in *pH* of pond water at L-Għadira ta' Sarraflu (SRF) ranging from 8.54 to 9.24 (mean: 8.95 ± 0.24) compared with coastal pools (9.23 ± 0.61) and inland pools (9.34 ± 0.87). Comparative data from coastal and inland wetlands collected by the authors during April and May 2020.
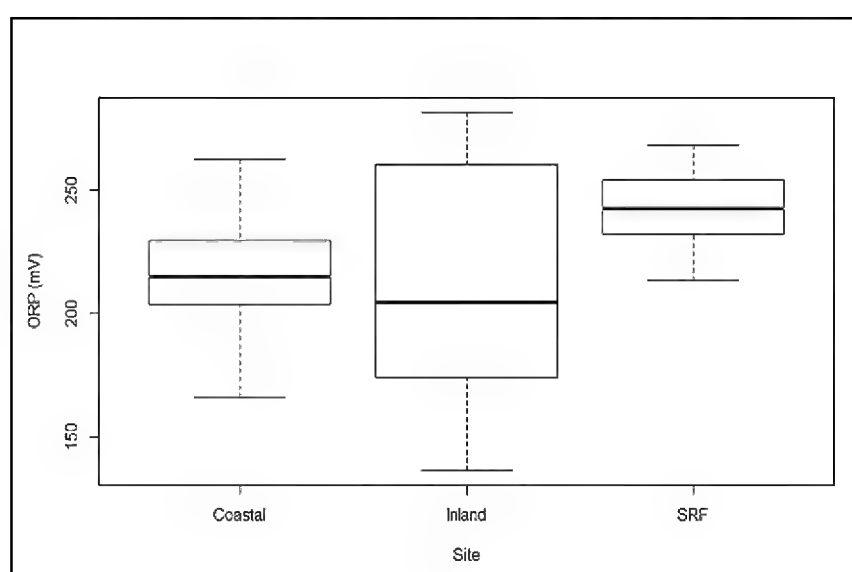
Figure 9. Relative variation in Oxidation-Reduction potential (ORP) of pond water at L-Għadira ta' Sarraflu (SRF) (242.38 ± 16.9mV) compared with that recorded from coastal pools (215.50 ± 23.59mV) and inland pools (211.67 ± 47.87mV). Comparative data from coastal and inland wetlands collected by the authors during April and May 2020.
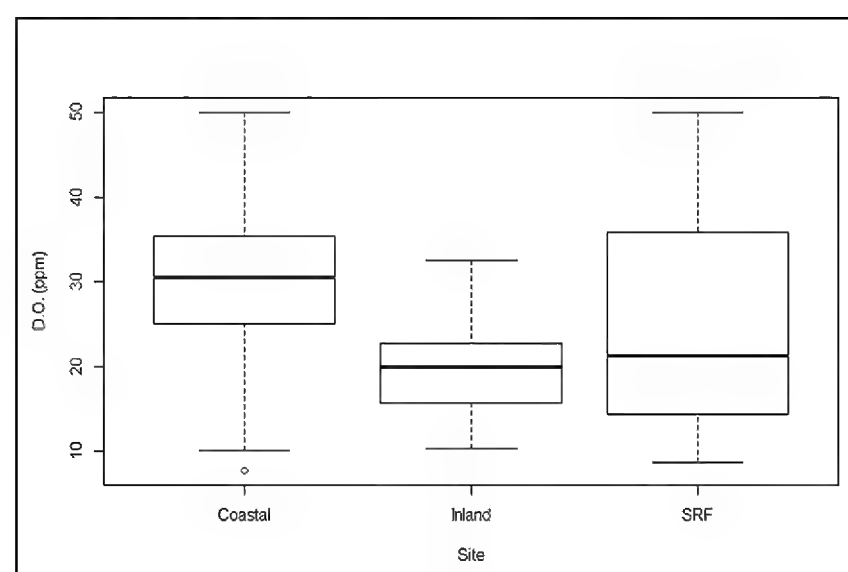
Figure 10. Relative variation in the dissolved oxygen (DO) concentration (ppm) of pond water at L-Għadira ta' Sarraflu (SRF) (25.30 ± 14.30ppm) compared with that recorded from coastal pools (31.20 ± 12.82ppm) and inland pools (19.53 ± 5.66ppm). Comparative data from coastal and inland wetlands collected by the authors during April and May 2020.

(Fig. 7). As a result, the EC range for SRF was found to be outside the range for "freshwater" given by the United States Geological Survey (USGS), and well within the "brackish-water" category. While the mean pH at SRF was found to be lower than the coastal and inland pools (Fig. 8), the mean ORP at SRF was higher (Fig. 9). Mean DO data from SRF however was comparable with that recorded from the coastal and inland pools (Fig. 10).

Identification of phytoplankton

Microscopic examination (x100) of unmodified pond water from SRF indicated a high-density

Figure 11. Variation of the concentration of *Peridinium* cells across samples. Mean density: 358118 ± 165227 cells mL⁻¹.
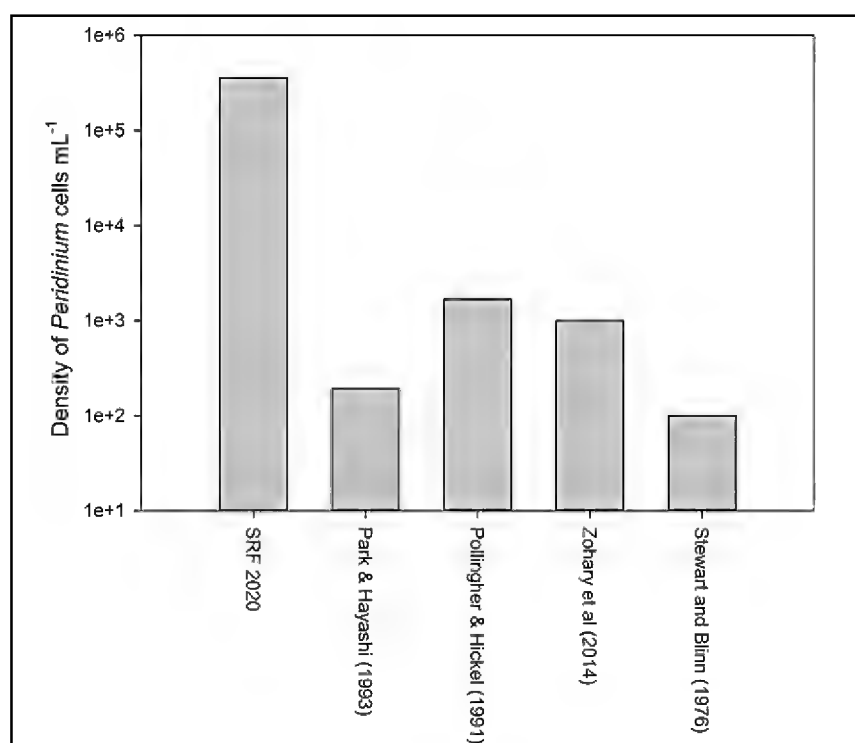
Figure 12. Comparison of the density in log scale of the *Peridinium* bloom at SRF with other blooms of the same genus recorded in literature. Bloom densities for *Peridinium* were obtained from Park & Hayashi (1993), Pollingher & Hickel (1991), Zohary et al. (2014) and Stewart & Blinn (1976).
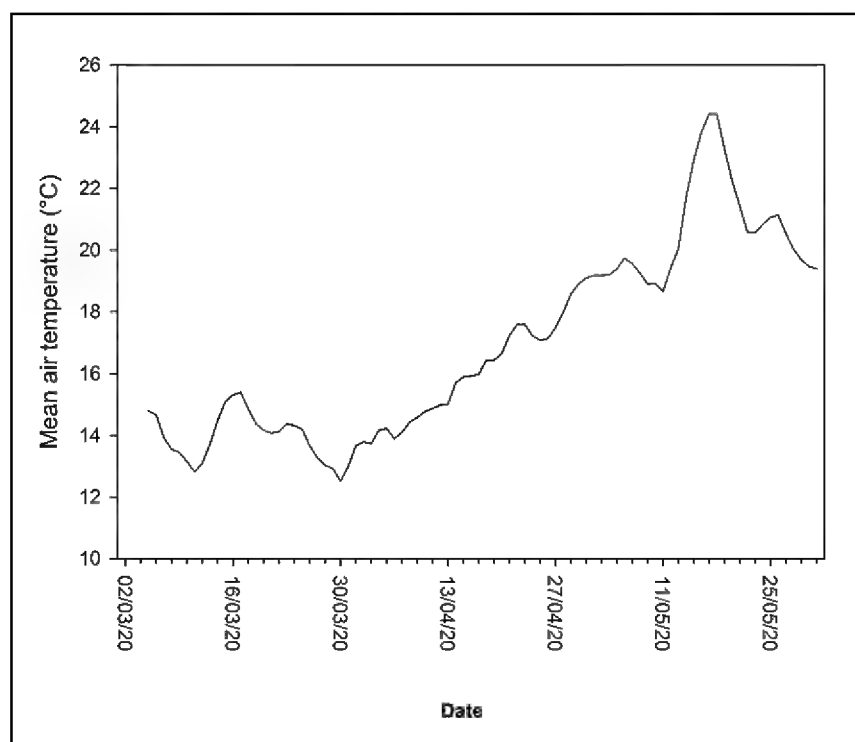
Figure 13. Mean air temperature (°C) during the period 1st March to 31st May 2020 expressed as a five-day moving average.

unialgal assemblage of ovoid free-swimming cells. The algal cells were observed to move in a 'whirling' pattern suggestive of dinoflagellates. Further examination of live cells under higher magnification (x400) indicated a transverse furrow traversing the central area of each cell as well as numerous brown chloroplasts. The shape of the cells, the position of the equatorial furrow and the numerous chloroplasts were consistent with the general

morphology of the genus *Peridinium* Ehrenberg, 1830. Cell diameters ranged from 11 μm to 41 μm (mean: 29.1 μm ± 5.54 μm), which were consistent with the size ranges for members of the genus in the literature (Carty, 2014; Cart & Parrow, 2015; Moestrup & Calado, 2018), and the configuration of the thecal plates was consistent with that of *Peridinium* cf. *cinctum* (O.F. Müller) Ehrenberg (Dinoflagellata Dinophyceae Peridiniaceae) (Kretschmann, 2020). Analysis of the micrographs also indicated the presence of other plankton in very low densities relative to the dinoflagellates, including rotifers and ciliates.

Density of **Peridinium**

The density of *Peridinium* cells per subsample ranged from 91 to 1185 cells, with an average of 465.3 ± 214.7 cells. This corresponded to 358118 ± 165227 cells mL^{-1} as indicated in figure 11. The density of the present bloom is put into context by comparing its cell density with that of other *Peridinium* blooms recorded in literature (Fig. 12).

Temperature data

The mean daily air temperature, as published by the Meteorological Office of Malta International Airport, showed a steady upward trend (Fig. 13) throughout April and early May 2020 (mean: 17.6 ± 2.8°C) relative to March 2020 (mean: 14.0 ± 1.3°C). The mean air temperature rose from 13.1°C on 3rd April to 18.1°C on 30th April, the day the bloom was first reported. It subsequently continued rising steadily, reaching 25.3°C on 15th May 2020. On the day of survey, the mean air temperature was 17.4°C, whilst the mean temperature of the pond water, measured just below the surface, was 21.2 ± 0.5°C.

DISCUSSION

Aside from being the first recorded bloom from this wetland, the cell density of *Peridinium* and its contribution to the total phytoplankton was prominent enough to attract and maintain public attention for several days. The study found that the density of the bloom was approximately between 200 to 3500 times higher than in others reported in the literature for the same genus (Fig. 12). Such phyto-

plankton density may however be a consequence of the restricted volume of SRF. As a result, the bloom cannot dilute with increasing distance from its origin as it would in a much larger body of water.

In the absence of previous records of blooms or water quality data for SRF, the underlying cause of the bloom could not be determined or placed into any meaningful context. Physicochemical parameters measured during the bloom therefore could not be compared against any mean values and variances as no baseline data was available. Consequently, only informed speculation could be drawn from the available data collected.

It should be noted that the mean air temperature increased steadily during the three weeks preceding the bloom and this was presumably also reflected in a corresponding rise in water temperature. Rapid increases in water temperature are known to be one of the possible drivers of algal blooms (Grigorszky et al, 2006) suggesting that this may have been a trigger for the SRF bloom. However, such temperature increases are part of an annual cycle, and they do not appear to have been accompanied by a bloom in previous years. The possibility that smaller, less detectable blooms did occur nonetheless exists.

The principal drivers of bloom events also include nutrient enrichment of pond water (Grigorszky et al, 2006). No information about the baseline nutrient status was available. Moreover, the nutrient levels of pond water during the bloom were not measured as the survey was carried out at a time when the Water Quality Laboratory at the University of Malta was not operational due to COVID-19 restrictions.

It is not known whether the *Peridinium* bloom at SRF was an isolated event as any previous blooms were either undetected or unreported. This highlights the need for a systematic monitoring programme of phytoplankton communities and water quality, as this would locate blooms such as this in a broader context and enable more effective management of this protected wetland.

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Emergence trap for woodpile insects provides two interesting species of Neuropterida from Sardinia

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ABSTRACT   An emergence trap for woodpile insects, assembled in the coppice holm oak forest of San Gregorio (SE-Sardinia), captured few specimens of only two species of Neuropterida, but these were very interesting ones: *Isoscelipteron glaserellum* (U. Aspöck, H. Aspöck et Hölzel, 1979) (Neuroptera Berothidae), new to Italy, and *Dendroleon pantherinus* (Fabricius, 1787) (Neuroptera Myrmeleontidae), new to Sardinia. Further specimens of both species were collected at the same site using other methods. An old find of *Dendroleon pantherinus*, previously considered unreliable, was confirmed. The ecology and rarity of both species are discussed.

KEY WORDS   *Isoscelipteron glaserellum*; Berothidae; *Dendroleon pantherinus*; Myrmeleontidae; geographic distribution.

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INTRODUCTION

Some insect species are considered rare because they are indeed rare in collections, but often their true rarity is questionable. Years ago, when defining pseudo-rarity, Gaston (1994: cfr. §2.5.2) wrote: "*Species may appear to be rare because methods appropriate to determining their true abundances and range sizes have not been applied, have not been applied in the right place, or have not been applied at the right time*". Confirming this obvious affirmation, much anecdotal evidence but few published papers (e.g., Duelli et al., 2006) exist for Neuropterida. Species living in the canopy, or having larvae which live concealed together with adults with a very short flying period, are typically apparently rare species. This pseudo-rarity is evident for adults which are not regularly captured using the more frequent sampling methods applied to Neuropterida (beating, sweeping, light-trapping). Methods applied for other insect orders sometimes permit collection of very interesting taxa among Neuropterida; a paradigmatic example is the discovery by Ghilarov (1962) from soil samples of the first Dilaridae larva living in soil.

Thanks to one of the authors (RR), an emergence trap for insects coming out from freshly cut firewood unexpectedly captured two species of Neuropterida new to Sardinia: *Isoscelipteron glaserellum* (U. Aspöck, H. Aspöck et Hölzel, 1979)

(Neuroptera Berothidae), and *Dendroleon pantherinus* (Fabricius, 1787) (Neuroptera Myrmeleontidae). These findings are reported here and integrated with other ecological and distributional data.

MATERIAL AND METHODS

The emergence trap derives from the prototype device described by Owen (1989, 1992): a device consisting in a pyramidal netting tent positioned on a woodpile. In our case the net was a readily-available bed mosquito net (Fig. 1). The woodpile is totally renewed each year in spring, is formed of freshly cut logs (average diameter 10 cm) of (prevailing) holm oak (*Quercus ilex* L.) and (occasional) strawberry tree (*Arbutus unedo* L.). The wood logs are consumed regularly during the cold season.

The trap was placed at the hamlet of San Gregorio, in the Sinnai council district, SE-Sardinia, 39°18'19"N 9°22'10"E. The dominant environment is a coppice holm oak forest. The trap was checked, day and night, at least once a week. It was set up in 2017.

Information about preparation, conservation and deposit collection are provided in the following sections, together with collection data.

RESULTS

Isoscelipteron glaserellum (U. Aspöck, H. Aspöck et Hölzel, 1979) Neuroptera Berothidae

EXAMINED MATERIAL. Specimens from emergence trap. * 22 June 2020, 1 male (Fig. 2) and 1 female, preserved in alcohol, R.A. Pantaleoni collection. * 21 September 2020, 1 female (Fig. 3), collected from the external side of the trap, preserved in alcohol, R.A. Pantaleoni collection.

Further specimens. * 19 July 2017, 1 specimen (very probably female), San Gregorio, Sinnai, SE-Sardinia, 39°18'19"N 9°22'10"E, about 100 m from the emergence trap, light trap, photographed but not collected (Fig. 4).

DISTRIBUTION. W-Mediterranean species known from Morocco (Aspöck U. & Aspöck H., 1991), Iberian Peninsula (Tillier et al., 2011; Monserrat, 2014), southern France (Mazel, 2004) and Corsica (Letardi et al., 2008). New to Italy.

REMARKS. *Isoscelipteron glaserellum* is rarely collected. Monserrat (2014), who summarizes the Iberian findings, cites just over 50 specimens, the vast majority of which were collected by means of light trap, one or two (occasionally three) specimens at a time. The captures took place in heterogeneous wooded habitats in Mediterranean environments.

The larval stage is known only for specimens reared from eggs from captive pregnant females (Monserrat, 2014). Therefore its bio-ecology is almost unknown. Larval termitophily and termitophagy is the most accredited hypothesis, but weakly demonstrated. This hypothesis is based on:

i) occasional findings of preimaginal stages of the Nearctic species of the genus *Lomamyia* Banks, 1904 near termite (particularly *Reticulitermes* Holmgren, 1913) nests (Gurney, 1947; Johnson & Hagen, 1981);

ii) exclusive acceptance of termites as prey in both *Lomamyia* and *Isoscelipteron* larvae (Tauber & Tauber, 1968; Brushwein, 1987; Monserrat, 2014), associated with a complex attack behaviour (Johnson & Hagen, 1981; Komatsu, 2014);

iii) perfect overlap of *Isoscelipteron* geographic ranges and *Reticulitermes* geographic ranges at least in the W-Palearctic region (compare maps in Austin et al., 2002, and Aspöck U. & Randolf, 2014).

The presence of termites in the woodpile can be excluded, whereas the presence under the woodpile can be neither confirmed nor excluded. The woodpile base is partly constituted by a dry stone wall: a termite nest deep in the soil is very unlikely, while the presence of termite tracks is possible. Unfortunately, the information provided by these findings does not help to clarify the biology of *I. glaserellum*, but rather broadens the hypotheses from which to start.

In any case, positive phototaxis of *I. glaserellum* is very occasional. One of the authors (RR) frequently performed light trapping sessions, often once a week at the site of the emergence trap, without further captures.

Dendroleon pantherinus (Fabricius, 1787) Neuroptera Myrmeleontidae

EXAMINED MATERIAL. Specimens from emergence trap. * 1 July 2017, 1 specimen, photographed but not collected (Fig. 5).

Figure 1. Emergence trap (2020 installation), San Gregorio, Sinnai, SE-Sardinia. Figure 2. *Isoscelipteron glaserellum* (Neuroptera Berothidae), male specimen in the emergence trap, 22 June 2020. Figure 3. Female specimen resting on the external side of the trap, 21 September 2020. Figure. 4. Specimen attracted by light, 19 July 2017. Figure 5, *Dendroleon pantherinus* (Neuroptera Myrmeleontidae), specimen found in the trap, 1 July 2017.

Further specimens. * 20 June 2004, 3 females, Cabras Lagoon [39°56'N 8°30'E], Oristano, Sardinia, collector unknown, pinned, R. Nicoli Aldini collection. * 17 July 2014, 1 male, San Gregorio, Sinnai, SE-Sardinia, 39°18'19"N 9°22'10"E [no further details known], collected by the late Francesco Rattu, pinned, R. Rattu collection.

DISTRIBUTION. European distribution, species absent from the Iberian Peninsula but present in the Caucasus (Aspöck H. et al., 2001), further records from Asia refer to closely related species (Krivokhatsky, 2011). In W-Mediterranean, known in France and Corsica (Colombo et al., 2013), and peninsular Italy and Sicily ([Bernardi] Iori et al., 1995). New to Sardinia.

REMARKS. *Dendroleon pantherinus* is widely distributed, although records are often occasional. Almost all captures occur in temperate broadleaved forests, a large percentage inside buildings located in the forests (Colombo et al., 2013). Only occasionally has it been collected in large numbers, i.e. in very favorable habitats (Duelli, pers. comm.; Pantaleoni, unpublished).

The first few collections of the larvae (Brauer, 1867; Roubal, 1936; Kelner-Pillault, 1958) occurred in old hollow trees, specifically in holes filled with dry detritus. Consequently, for many years this was considered its exclusive habitat. Mature broadleaved forests, rich in old trees, appeared to be the preferred habitat of the adults too. However, in a survey of 183 hollow trees *D. pantherinus* was registered in only 11 cases (Colombo et al., 2013).

Steinmann (1967) was the first author to attribute wider ecological range to the larvae of this species. Still, his statement that larvae can live in street dust ('*utca poráfan*') was neglected at least until the behavioural synanthropy of this species was demonstrated by Gepp & Hölzel (1989) and Gepp (2010). The larvae are able to live in house dust, in carpets and other unexpected situations. Exploring an abandoned house (24 July 2010, Campigno, Marradi, Florence, Tuscany, 44°01'28.97"N 11°35'17.77"E) in which an adult of *D. pantherinus* was collected, one of the authors (RAP) found I and II instar exuviae of this species in the ash of a firebox, enriched by bat feces dropping throughout the fuel. This disaggregated material on the hearth hosted many saprophages, such as Dermestidae and Tenebrionidae larvae.

The larvae have also been collected under bark (Tröger, 1999). Two further unpublished reportings of this behavior are available:

i) 26 March 2013, a larva collected under *Quercus suber* L. bark by Marco Bastianini and Stefano Nappini, Bandite di Scarlino, Grosseto, Tuscany, Italy;

ii) 26 March 2015, a larva collected under oak bark in a mixed (*Tilia*, *Carpinus*, *Quercus*, *Fagus*, *Fraxinus*, *Ulmus*) broadleaved forest by Alexandru Mihai Pintilioaie, near Tomesti, Iasi, Moldova, Romania.

The capture using an emergence trap in Sardinia is probably also due to a mature larva living under the bark of the logs in the woodpile.

The presence of *D. pantherinus* in Sardinia must be wider than suspected. The specimens from Oristano remained for years in the collection of RNA considered as not being fully reliable, due to the unknown collector and the environments near the capture locality. However, the new findings in SE-Sardinia have added evidence supporting the reliability of this old finding.

In conclusion, *D. pantherinus* is preferentially associated with mature broadleaved forests, but is able to adapt to many wooded habitats, from coppices to urban parks (our colleague Maurizio Pavesi found specimens in the attic of the Museo Civico di Storia Naturale in Milan, which is located in an area - 'Giardini Pubblici di Porta Venezia' - characterized by the presence of many trees). The motionless larvae are ambush predators, staying concealed with their bodies covered by debris, in a variety of microhabitats, including anthropogenic ones. The scarcity of this species in collections is probably more due to the adult behavior, such as remaining in the canopy or weak positive phototaxis, than to a true rarity.

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New records of the reed cricket *Natula averni* (Costa, 1855) (Orthoptera Gryllidae) in Sicily

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ABSTRACT   Three new distribution records of the reed cricket *Natula averni* (Costa, 1855) (Orthoptera Gryllidae) are reported for Sicily based on sound recordings. Additional information is provided for the song of this rare cricket species.

INTRODUCTION

Knowledge about the distribution and ecology of the reed cricket *Natula averni* (Costa, 1855) (Orthoptera Gryllidae) is rather poor. Italian records for the species are rare. After the description of the species in 1855 from near Naples (Costa, 1855) the species was detected in Sardinia in 1987 (Schmidt & Herrmann, 2000) and in Sicily in 2006 (Odé et al., 2011). Some records are also known from the provinces of Apulia, Basilicata, Calabria and Tuscany (Island of Elba).

The situation on Sicily can be explained as follows. The first find on the basis of sound recordings was at the Foce del Belice by Odé et al. (2011). A second locality has been photographically secured by Luigi Barraco near Trapani and published by Surdo (2019). Odé et al. (2011) put forward a call for extra information and as our summer holiday in 2020 was held on Sicily, I was willing to respond to the call and take up the challenge.

MATERIAL AND METHODS

The well-known locality at the Foce del Belice

(mouth of the river Belice) was visited to build a good reference to the sound. The animals were heard singing in the evening of August 2 and the morning of August 3, 2020. A couple of localities with circumstances that seemed to fulfill the species' needs, mainly the mouths of perennial current streams, were planned to visit. They concerned the mouths of the rivers (foce del fiume) Modione, San Leonardo, Tellaro and Simeto.

Recording equipment consisted of a mono microphone (Clippy EM172) and an Edirol R-09 digital recorder. The microphone broke down after the first location and was later replaced by a brandless stereo microphone. Eventually the use of mobile phones (Samsung Galaxy A5 with the Hi-Q mp3 recorder app and I-phone 7, both with internal microphones) was added to record songs.

For analysis and graphic production, the software program Elekon Batexplorer Version 2.1.7.0 and the free sound editing software of Ocenaudio have been used to generate oscillograms and spectrograms. As the song of this cricket appeared to be within a narrow band of frequencies around 6-8 kHz, a high pass filter with a cut-off frequency of just below the echeme frequency has been used to clean the recordings from disturbing noises (wind, breakers, birds

and people). With a low pass filter sometimes the song of a second male at a slightly higher frequency could be cleaned off too. Some characteristics of the song were compared with the literature.

RESULTS

Attempts to reach the river San Leonardo (from the north) within hearing distance failed, due to gated ways. At the other three river mouths the presence of reed crickets was detected immediately at arrival. Silent moments without singing males were not registered. The three new records are presented on the map in figure 1 together with the two previously identified localities.

Exact locations, dates, availability of recordings and used equipment are presented in Table 2.
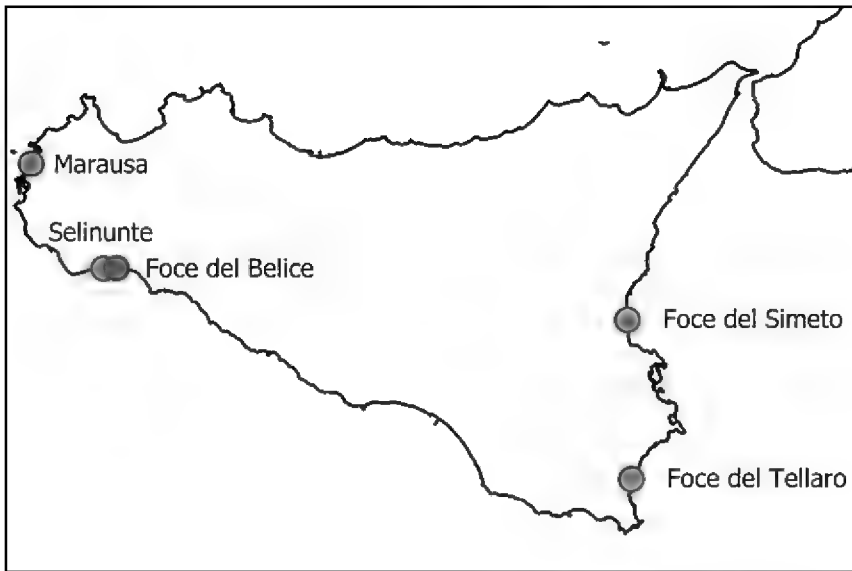
Figure 1. Map of the distribution of *Natula averni*. Light blue: Foce del Belice; yellow: Marausa; red: new records.

Only bioacoustic evidence was collected. Sightings of specimens did not work out, despite occasional occurrence of singing males within very short distance.

Due to a technical failure of the microphone no sound recordings are available from Foce del Modione. Some sound characteristics of males singing at the Belice, Tellaro and Simeto river mouths are given in Table 1 and compared to the Sicilian track from Odé et al. (2011). In figures 2 and 3 some of the species-specific sound characteristics are shown graphically (oscillogram and spectogram).

The Modione population, within the archaeological site of Selinunte, is separated from the Belice population by almost 4 km of unsuitable habitats. The Tellaro and Simeto river mouths are located 215 and 197 km from Belice respectively.

Though different between recordings, within each recorded song the number of syllables per echeme was remarkably constant (see ranges and very low standard deviations). The recorded sound characteristics were found to be largely consistent with the data given by Odé et al. (2011). Some deviations were noticed and are treated in the discussion.

DISCUSSION

With relatively little effort, three new distributional records could be added to the only two populations known up until now. Two of the new records are from Syracuse and Catania provinces at the east side of the island far outside the distri-

	temp. (°C)	evaluated number of echemes	mean echeme duration (ms, min-max)	echeme tempo (/s)	syllable tempo (/s)	mean number of syllables per echeme ±SD (min-max)	peak frequency (kHz)
Belice*	25	?	240 (230–250)	2.2	87	20.9 (20–21.8)	6.4
Belice	32	23	223 (197–232)	2.4	96	21.4±1.1 (19–23)	7.1
Tellaro	24	20	326 (310–347)	2.1	80	26.1±1.2 (24–29)	6.7
Simeto 1	26	11	245 (234–296)	3.0	125	30.6±2.6 (26–35)	7.5
Simeto 2	26	8	273 (263–283)	2.7	64	23.9±1.4 (22–27)	6.8

Table 1. Details of sound recordings. *: Take 33 as recorded by B. Odé (Odé et al., 2011).
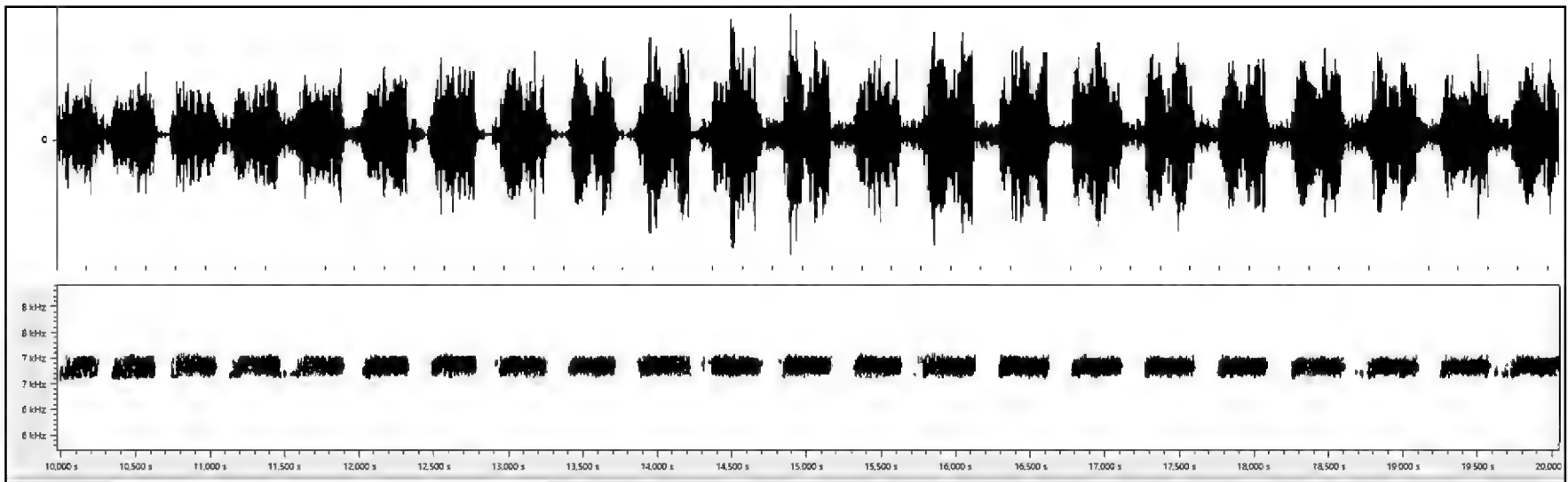
Figure 2. Oscillogram and spectogram of 10 sec. of a song at Foce del Tellaro on August 7, 2020.
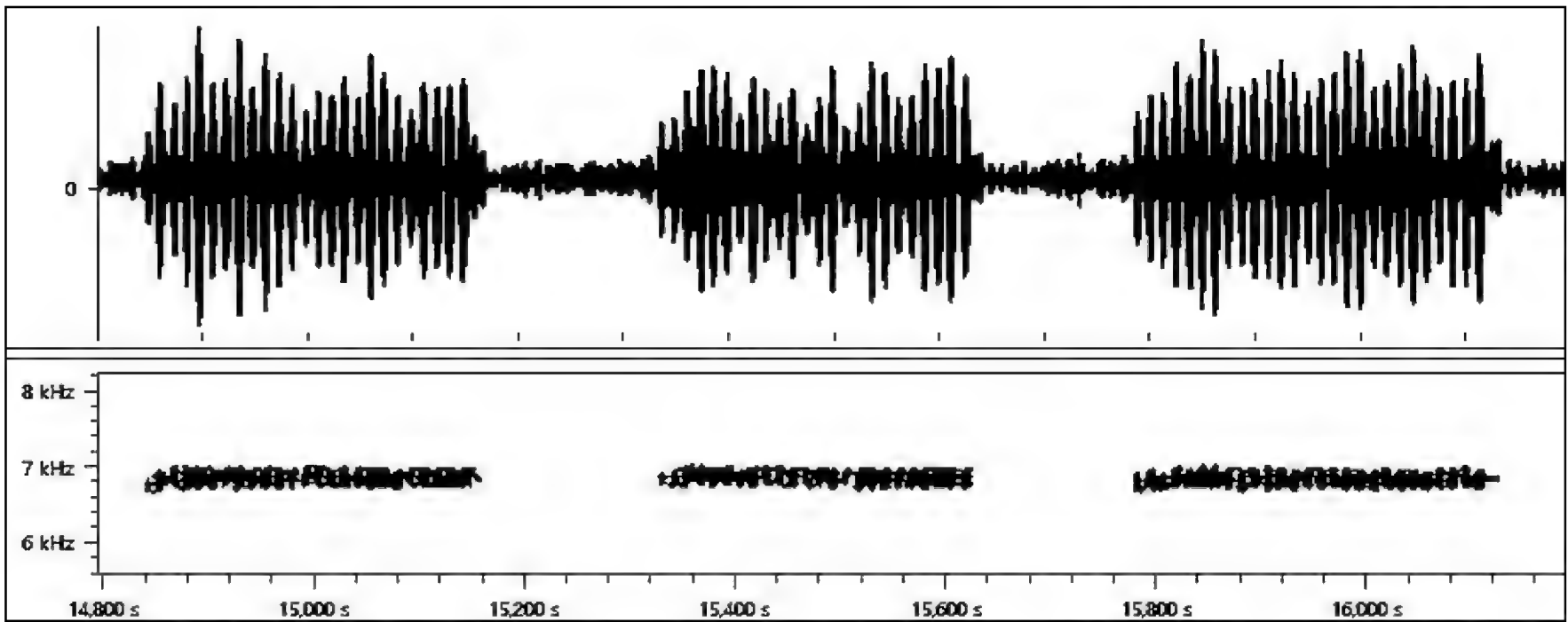
Figure 3. Oscillogram and spectogram of 2 sec. of a song at Foce del Tellaro on 7 August 2020.

River	Municipality/Province	Coordinates	Date	Recording method
Belice	Castelvetrano/Trapani	37.583118N 12.866478E	2.VIII.2020	24 bit wav, 44.1 kHz, mono microphone
Modione	Castelvetrano/Trapani	37.582430N 12.822300E	3.VIII.2020	not available
Tellaro	Noto/Syracuse	36.837880N 15.105492E	7.VIII.2020	24 bit wav, 44 kHz wav, Hi-Q mp3 rec app, mobile phone Samsung Galaxy A5 & I-phone 7, internal microphone
Simeto	Catania/Catania	37.400670N 15.088050E	8.VIII.2020	24 bit wav, 44.1 kHz stereo microphone

Table 2. Data on locations and recordings.

bution known, which makes it probable that the species has a large distribution, maybe all around the island. It is suspected that the distribution is highly underreported and several other populations must exist. The San Leonardo river, that could not be reached, Lago Gornalunga close to the Simeto river and several other river mouths like those of the rivers Platani, Salso, Anapo, Cassabile, Asinaro, Irminio are all good candidates and are in need of exploration.

Although no sightings or voucher specimens could be obtained, the unequivocal identification was possible by audible perception of the very specific sound and digital recordings thereof.

As the population density was high, multiple males were always singing simultaneously and getting clear sound recordings in situ proved difficult. Most recordings show (traces of) other singing males, which can be seen as vague echemes on slightly different frequencies (from a second male nearby) in the spectograms, noise at the specific peak frequency or noise between echemes and in the low dB-range (from other males further away). The Simeto1 recording showed a remarkable high syllable tempo (125 instead of 80–100) together with a high number of syllables per echeme with a mean of 30 instead of 17–21.

Equally loud syllables within echemes, as described by Odé et al. (2011), was not the rule in this study. The same authors also wrote that sometimes the first 1–3 syllables are weaker than the following ones. In this study this was found in some cases, but in addition it was noticed that the narrow frequency band also raised with approximately 0.1 kHz during this start before getting a quasi constant frequency (Fig. 3).

According to Odé et al. (2011) the song could be heard in the afternoon, evening and night. In this study songs were also heard and recorded in the mornings at all four localities up to four hours after sunrise and later observations only lack due to leaving the area. The song probably can be heard throughout the day, which facilitates research.

Except for the non-important harmonics, the song of the reed cricket does not contain ultrasonic components, which means that relative simple recording equipment can be used to provide proof of presence.

ACKNOWLEDGEMENTS

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A new subspecies of *Carabus* (*Cratocephalus*) *solskyi* Ballion, 1878 from Tianshan Mountains, Xinjiang province, Northwest China (Coleoptera Carabidae)

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ABSTRACT   A new subspecies of *Carabus* (*Cratocephalus*) *solskyi* Ballion, 1878 from Xinjiang Province, Northwest China is described and figured.

KEY WORDS   Coleoptera; Carabidae; *Carabus*; *Cratocephalus*; new subspecies; Xinjiang; China.

INTRODUCTION

Through the courtesy of Mr. Chengyi Zhao (Guangzhou, China), we recently had an opportunity to examine a short series of *Carabus* (*Cratocephalus*) *solskyi* Ballion, 1878 collected near Kashi, southeast from Yining in Xinjiang Province.

These specimens are well discriminated from all the hitherto known races of the species, particularly by *C. solskyi solskyi* and *C. solskyi toropovi* Obydov (1997).

We are describing them as new subspecies in the following lines.

RESULTS

Systematics

Ordo COLEOPTERA Linnaeus, 1758
Subordo ADEPHAGA Schellenberg, 1806
Familia CARABIDAE Latreille, 1802
Subfamilia CARABINAE Latreille, 1802
Genus *Carabus* Linnaeus, 1758
Subgenus *Cratocephalus* Kirsch, 1859

***Carabus* (*Cratocephalus*) *solskyi chengyii* n. ssp.**
(Figs. 1–4) - http://zoobank.org:act:97EBA6E9-5290-470A-8B44-EA8272E6A925

EXAMINED MATERIAL. Holotype male, China, Xinjiang province, Yining County, Kashi Township Reservoir, VII.2020. The holotype is temporarily housed at the author's collection (Ivan Rapuzzi, Prepotto, Italy) waiting to be definitively deposited in a public Institution. Paratypes: 1 male and 2 females, same data as the holotype, preserved in the author's collections.

DESCRIPTION OF HOLOTYPE. Length including mandibles 30 mm (Fig. 1), maximum width of elytra: 11.7 mm. Upper surface with strong metallic lustre, very shiny: elytra green; pronotum black, largely violet margined; black head; ventral surface, legs, palpi, antennae, and mandibles black. The head is stout but somewhat smaller and more elongated, frons flattered; mandibles broader and very elongate, apex strongly acuminated; the left one with a slightly prominent obtuse basal ridge. Second antennal segment strongly depressed. Hind angles of pronotum forming long and triangular lobes,

longer prominent and bent downwards. Very rough and wrinkled elytral sculpture, stronger and deeper foveae with strong green metallic lustre; all intervals disrupted. Male aedeagus typical of the species; more regularly curved in frontal view (Fig. 2), lateral view (Fig. 3). Legs of normal length, the fourth segment of male protarsi is dilated with the ventral side strongly pubescent.

VARIABILITY. The paratypes have no substantial morphological differences with the holotype. The females (Fig. 4) are of slightly larger size (31 to 33 mm); the body shape is larger and more rounded.

ETYMOLOGY. The new subspecies is named in honour of Mr. Chengyi Zhao (Wuxi, China), lucky collector of the type specimens.

DISTRIBUTION. *Carabus* (*Cratocephalus*) *solskyi sensu lato* is widespread in a large area of Central Tianshan and Borohoro Mountains (Deuve, 2013). Through its areal the species has a scarce variability, *C.* (*Cratocephalus*) *solskyi toropovi* was established upon specimens from the Northwest limit of the species (Bakanas, Ili river, North from Almaty, Kazakhstan). The new subspecies seems isolated on the hills on the east side of Kax River valley that well divides its areal from the typonominal subspecies.

Figures 1–3. *Carabus* (*Cratocephalus*) *solskyi chengyii* n. ssp. holotype (Fig. 1) with aedeagus in lateral view (Fig. 2) and aedeagus in dorsal view (Fig. 3). Figure 4. *Carabus* (*Cratocephalus*) *solskyi chengyii* n. ssp. paratype female.

REMARKS. Morphologically, *C.* (*Cratocephalus*) *solskyi chengyii* n. ssp. is easily distinguished from *C. solskyi solskyi* and *C. solskyi toropovi* by strong metallic lustre of elytra and pronotum, shorter and rounded body shape, smaller and flattered head, longer and narrower mandibles, and stronger and wrinkled elytral sculpture (see also Obydov, 1997; Deuve, 2013).

ACKNOWLEDGEMENTS

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New data on *Acicula benoiti* (Bourguignat, 1864) (Gastropoda Architaenioglossa Aciculidae) and description of *A. hierae* n. sp. from Marettimo Island (Sicily, Italy)

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ABSTRACT   New morphological and distributional data on *Acicula benoiti* (Bourguignat, 1864) (Gastropoda Architaenioglossa Aciculidae) in Sicily are provided. In addition, *A. hierae* n. sp. from Marettimo Island (Egadi Islands, N-W Sicily, Italy) is here described. The new species is characterized by conic-subcylindrical shape, with obtuse apex, thick parietal callus; well developed angular tooth; external peristomal varix raised, rounded, and anteriorly and posteriorly delimited by simple line.

KEY WORDS   *Acicula*; land snails; Sicily; biodiversity; taxonomy; island endemism.

INTRODUCTION

The small operculate prosobranch gastropods of the genus *Acicula* W. Hartmann, 1821 are widespread in the western Palaearctic Region (MolluscaBase, 2020). Currently, in Sicily only a species is known, *A. benoiti* (Bourguignat, 1864) (Boeters et al., 1989; Bodon et al., 1995; Reitano et al., 2009, 2012; Bank & Ramos, 2017; MolluscaBase, 2020).

Field surveys carried out in recent years in Sicily and surrounding islands allowed us to increase knowledge on the genus *Acicula* in this region.

In this paper, new data on *A. benoiti* and the description of a new species from Marettimo Island, *A. hierae* n. sp., are provided.

RESULTS

Empty shells and live specimens were collected in the field on sight on the soil and under the rocks or by sieving litter and soil. Observations on ecology of these organisms were made directly in the field, and in the laboratory where living snails were bred in plastic boxes. These little terrariums (5 cm x 6 cm x 5 cm) contained a thin layer of autochthonous topsoil, litter of native plant and small fragments of carbonate rock; the humidity was kept high and the contents were changed twice a week. The empty shells have been immersed for less than two minutes in hydrogen peroxide (H_2O_2) and than in sodium hypochlorite NaClO, and subsequently cleaned with water and a flat bristle brush. Dry shells have been studied regarding size, colour, morphology, sculpture.

In order to study and illustrate genital organs, some specimens were drown in water and fixed in 80% ethanol. The bodies were isolated from the shells and dissected under the Optika stereomicroscope using a very fine pointed scalpel, scissors and needles. Photos were taken with a Canon EOS 100D

camera and Laowa 2.5x5Xultra macro. Specimens were also examined uncoated under a Tescan Vega 2 LMU Scanning Electron Microscope in Low Vacuum modality to investigate tube micromorphology. All images were acquired at the Department of Biological, Geological and Environmental Sciences (University of Catania). Height and maximum diameter of the shell along with some parts of genitalia were measured (in millimeters) by a digital gauge. Taxonomical references are based on the checklist of the land and freshwater Gastropoda of Europe (Bank & Ramos, 2017; MolluscaBase, 2020).

The materials used for this study are deposited in the following Museums and private collections: F. Liberto collection, Cefalù, Italy (CL); Museo Civico di Zoologia, Rome, Italy (MCZR); A. Reitano collection, Tremestieri Etneo, Catania, Italy (CR), I. Sparacio collection, Palermo, Italy (CMS); R. Viviano collection, Palermo, Italy (CV).

ABBREVIATIONS AND ACRONYMS. D: maximum diameter; ex: specimen/s; E: eye; F: foot; H: shell height; LWH: height last whorl; m: meter/s; mm: millimeter/s; O: operculum; P: penis; sh: shell/s; T: tentacle.

RESULTS

Systematics

Phylum MOLLUSCA Cuvier, 1795
Classis GASTROPODA Cuvier, 1795
Subclassis CAENOGASTROPODA Cox, 1960
Ordo ARCHITAENIOGLOSSA Haller, 1890
Superfamilia CYCLOPHOROIDEA Gray, 1847
Familia ACICULIDAE Gray, 1850
Genus *Acicula* W. Hartmann, 1821

Acicula benoiti (Bourguignat, 1864) Figs. 1–4, 7
htpp://zoobank.org:act:34A7EC67-59ED-489D-9ABB-733F074D2DB5

Pupula lineata - Calcara, 1845: 34 - *nel fiume Oreto, presso il ponte delle Grazie, alla Guadagna, ed a Mondello* - (non Draparnaud, 1801)
Acme lineata - Benoit, 1859: Pl. 5, Fig. 33 (not Draparnaud, 1801)
Acme benoiti - Bourguignat, 1864: 218–219 - *Sicilie*
Acme benoiti - Paladilhe, 1868: 323 - *Sicilie, environs de Palerme*

Acme benoiti - Westerlund, 1871: 424 - *Sicilien*
Acme lineata - Benoit, 1875: 154 - *alle sponde del fiume Oreto, sotto il ponte delle Grazie* (not Draparnaud, 1801)
Acme benoiti - Schacko, 1875: 147–148, Pl. 5, Fig. 4 - *Sicilien*
Acicula benoiti - Pfeiffer, 1876: 5 - *Sicilia*
Acme benoiti - Paulucci, 1879: 192–194 - *Sicilia*
Acme benoiti - Kobelt, 1881: 131 - *Sicilien*
Acme benoiti - Paulucci, 1881: 223 - *Sicilia, Corleone*
Acme lienata - Benoit, 1882: 149–150 - *campagne di Palermo, alle sponde del fiume Oreto, sotto il ponte delle Grazie* (not Draparnaud, 1801)
Acme (Acicula) benoiti - Westerlund, 1885: 101 - *Sicilien bei Palermo*
Acme lineata var *subdiaphana* - De Gregorio 1895: 205 - *Fiume Oreto (Palermo)*
Acme benoiti - Kobelt in Rossmaessler, 1896: 10, Pl. 182, Fig. 1150 - *in der Umgebung von Palermo*
Acme (Auricella) benoiti - Kobelt & Mollendorff, 1897: 74 - *Sicilien*
Acme benoiti - Kobelt, 1898: 298 - *Sicilien*
Acme (Auricella) benoiti - Kobelt, 1908: 166 - *Palermo*
Pupula benoiti - Hesse, 1920: 88 - *Palermo*
Acicula benoiti - Gittenberger & Boeters, 1977a: 218–219
Acicula benoiti - Boeters et al., 1989: 27, 28, 30–32, 34, 54, 72 Figs. 21, 25, 26, 54 - *Sizilien, in den Provinzen Palermo und Siracusa*
Acicula benoiti - Bodon et al., 1995: 20 - *Sicilia*
Acicula benoiti - Cossignani & Cossignani, 1995: 38, 39 - *Sicilia*
Acicula cfr. *benoiti* - Reitano et al., 2009: 184, Fig. 4a-b - *Melilli, Grotta Palombara, in ambiente ipogeo*
Acicula benoiti - Liberto et al., 2010: 38, Fig. 24 - *Sicilia*
Acicula benoiti - Reitano et al., 2012: 558–559, Fig. 9 - *Palermo, Grotta Conza*
Acicula benoiti - Welter-Schultes, 2012: *Syracuse*
Acicula benoiti - Cossignani & Cossignani, 2020: 18, 19 - *Palermo*
Acicula benoiti - MolluscaBase 2020 - *Sicily*

EXAMINED MATERIAL. Italy, Sicily, Palermo, Fiume Oreto presso Ponte della Grazia, 38°04'44.6"N 13°19'22.9"E, 80 m, 18.XI.1990, 6 sh, (CMS 1258/6); Palermo, Grotta Molara, 38° 8'47.30"N 13°18'17.58"E, 117 m, 12.VIII.2002, 2 sh (CR)
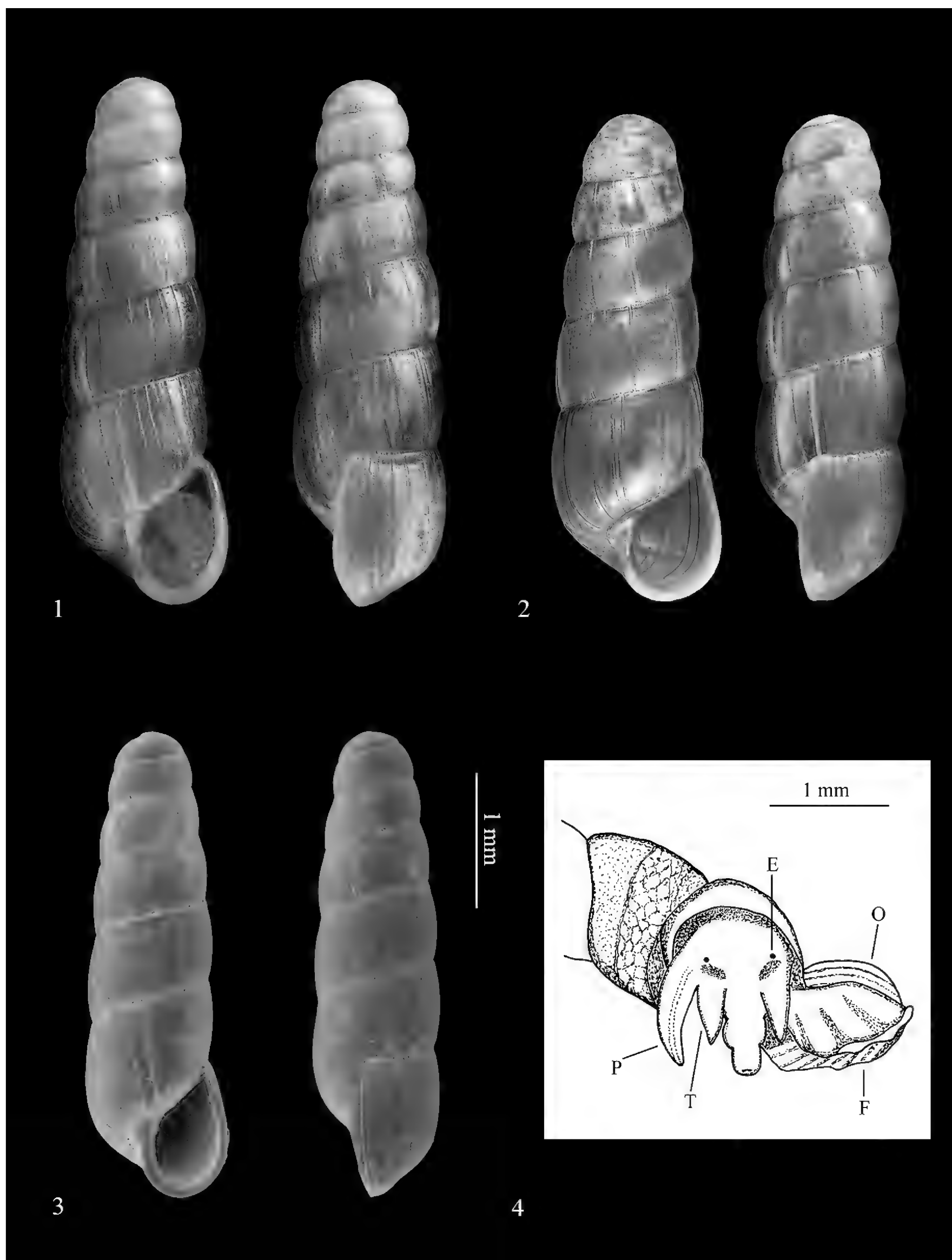
Figures 1–4. *Acicula benoiti.* Fig. 1: Italy, Sicily, Palermo, Fossa della Garofala, H: 4.1 mm, with soft part. Fig. 2: Idem, H: 3.8 mm. Fig. 3: Italy, Sicily, Palermo, Grotta Molara, H: 3.4 mm, SEM photo. Fig. 4: Body of *Acicula benoiti* from Palermo, Fossa della Garofala.

(Fig. 5); Gratteri, Pizzo Giurafelle, 37"58'19°N 14°00'08"E, 767 m, 14.X.2007, 9 sh (CL 3162–3170); Gratteri, Pizzo Giurafelle, 37°58'21"N 14°00'05"E, 700 m, 19.I.2008, 8 sh (CL 3380–3387); Carini, Monte Columbrina, Grotta Za Minica, 38°09'29"N 13°13'43"E, 18.VIII.2008, 7 sh (CL 3542–3548); Gratteri, Vallone tra Rocca Stefana e Pizzo Giurafelle, 37°58'54"N 14°00'20"E, 650 m, 2.XI.2008, 2 sh (CL 4083–4084); Palermo, Grotta Conza, 38°11'14"N 13°16'42"E, 190 m, legit S. Giglio, V.2012, 1 sh (CL 17325); Gratteri, Monte Dipilo, Pizzo Dipilo, 37°56'55"N 13°58'47"E, 1134 m, 17.XI.2013, 2 sh (CL 6260–6261); Palermo, Bellolampo, Pizzo Capra, east slope, 38°07'58"N 13°16'39.5"E, 355 m, 03.V.2015, 1 sh (CV); Palermo, Monte Gallo, La Fossa, 38°12'02"N 13°17'59"E, 92 m, IV.2016, 1 ex (CV); Palermo, Monte Castellaccio, 38°09'36"N 13°15'50"E, 800/850 m, 26.V.2016, 4 sh (CV); Palermo, Inserra, Cozzo San Croce, 38°08'57"N 13°17'37"E, 400 m, 07.VI.2016, 1 sh (CV); Palermo, Monte Pellegrino, Pizzo Monaco, 38°10'11.5"N 13°21'33"E, 485 m, 13.VI.2016, 4 sh (CV RV19–0411); Palermo, Pizzo Manolfo, 38°10'13"N 13°16'25"E, 615 m, 16.X.2016, 1 sh (CV); Altavilla Milicia, Grotta Mazzamuto, 38°01'24.3"N 13°33'45"E, 195 m, 01.XII.2018, 1 ex (CV); Cinisi, Monte Pecoraro, Cima Bosco Tagliato, north slopes, 38°10'08"N 13°07'35"E, 450/520 m, 10.II.2019, 13 sh (CV); Palermo, Monte Pellegrino, Addaura, 38°11'08"N 13°20'43"E, 145/155 m, 06.IV.2019, 9 sh (CV); Palermo, Fossa della Garofala, 38°06'04"N 13°20'40"E, 50 m, 24.IV.2019, 26 sh (CV) (Fig. 2); Palermo, Fossa della Garofala, 38°06'04"N 13°20'40"E, 50 m, 24.IV.2019, legit R. Viviano, 3 sh (CMS 5251/3) (Figs. 1, 4); Palermo, Monte Pellegrino, Roccia dello Schiavo, west slopes, 38°09'40.7"N 13°20'44"E, 5 sh, 110–150 m, 20.V.2016 (CV RV19–0225); Palermo, Monte Gallo, La Fossa, 38°12'32.6"N 13°17'29.6"E, 170–185 m, 30.V.2020, 4 sh (CV).

Description. Shell (Figs. 1–3) dextral, elongate, conic-subcylindrical with obtuse apex, H: 3–4.6 mm, D: 1–1.3 mm; LWH: 1–0.9 mm, glassy and sub-transparent, light brown in color with a thin subsutural darker brown band. Spire consisting of 6½–7 ½ slightly convex whorls; suture shallow. The last whorl is 22–27 % of the total shell height. External surface of teleoconch smooth, with irreg-

ular spaced radial grooves, 10–20 in penultimate whorl. Aperture oval-pyriform, wide; large columellar callus; peristoma edge almost straight in lateral view; evident angular tooth; external peristomal varix swollen; in lateral view it has the same width troughout its lenght, more flat above and swelling in the basal part where it appears without clear demarcation.

Operculum semi-transparent, very thin, paucispiral with semi-central nucleus; the outer face shows growth lines (10–12) higher in the central part; the inner face is smooth.

White body, two thin blackish spots at the base of the tentacles, black eyes, foot with whitish sole; whitish visceral sac with yellowish spots mainly gathered on the penultimate whorl. The margin of the mantle partially covers the head; two tentacles wider at the base, and with rounded apex, shorter in the males and thinner and more elongated in the females. The penis is conic elongated, subelliptical in cross section, slightly curved, slightly enlarged at the base and tapered near apex which is flattened, about a third longer than the tentacles; it is crossed by a groove over the entire ventral surface up to the apex (Fig. 4).

Distribution and Biology. *Acicula benoiti* is an endemic Sicilian species. According to the data in the present work, it is widespread especially in north-western Sicily: Mountains of Palermo, Madonie and Sicani Mountains, while no data are known for the mountains of Trapani (Fig. 7).

It is also reported for south-eastern Sicily (Syracuse province) by Boeters et al. (1989) and Welter-Schultes (2012). The report from Grotta Palombara, Syracuse, is only for comparison (Reitano et al., 2009: *A.* cfr. *benoiti*). Fiorentino et al. (2004) report *A. benoiti* for Marettimo island, but we believe that this record should be referred to new species that we describe below.

Acicula benoiti has been found in the bedding at the base of trees and large bushes of the Mediterranean scrub, even in partially naturalized crops (*Quercus ilex* L., *Euphorbia dendroides* L., *Pistacia lentiscus* L., *Fraxinus ornus* L., *Rhamnus alaternus* L., *Laurus nobilis* L., *Celtis australis* L., *Morus alba* L., *Hedera helix* L., *Acanthus* sp., *Citrus* spp., *Rubus* sp.). It lives at the base of shady limestone walls, in endogeous environment, under stones and debris, always in damp and shady environments.

STATUS AND CONSERVATION. *Acicula benoiti* is "Vulnerable", according to Cuttelod et al. (2011) and Neubert et al. (2019).

REMARKS. *Acicula benoiti* was described by Bourguignat (1864) for Sicily ("*Habite la Sicile*") based on specimens received by Benoit ("*in Specim. Ab illo missis, in the year 1857*"). The Sicilian author (Benoit, 1882) had collected these specimens "*nelle campagne di Palermo, alle sponde del fiume Oreto, sotto il ponte delle Grazie*" [in the countryside of Palermo, on the banks of the Oreto river, under the Ponte delle Grazie] reiterating that the specimens described by Bourguignat came from these localities.

Boeters et al. (1989) based on this reconstruction, designate the lectotype and 4 paralectotypes from "*Palermo, Oreto-Genist*".

Acicula hierae n. sp. (Figs. 5–7)

TYPE LOCALITY. Marettimo Islands (Egadi Islands, north-western Sicily, Italy).

TYPE MATERIAL. Holotype (MCZR-M-TYPE 00252): Italy, Sicily, Marettimo Island, Canalazzo, 37°58'20"N 12°03'33"E, 420 m, 22.IV.2018, legit R. Viviano (Fig. 4). Paratypes: Italy, Sicily, Marettimo Island, Case Romane, 37°58'14"N 12°03'51"E, 230/240 m, 28.VII.2015, 1 sh (CV); Carcaredda, 37°57'12"N 12°04'40"E, 140/160 m, 24.I.2016, 2 sh (CV); Carcaredda, 37°57'16"N 12°04'30"E, 200 m, 10.III.2019, 3 sh (CR); Canalazzo, 37°58'20"N, 12°03'33"E, 426 m, 24.I.2016, 1 sh (CL 16417); trail above Case Romane, 37°58'14.3"N 12°03'39.7"E, 318/350 m, 22.IV.2018, 1 sh (CV); Canalazzo, 37°58'20"N 12°03'33"E, 420 m, 22.IV.2018, 2 sh (CV); trail to punta Ansini, 37°58'06"N 12°03'44.7"E, 375 m, 08.III.2019, 1 sh (CV); surroundings of Semaforo north slope, 37°57'37"N 12°03'41"E, 380/400 m, 08.III.2019, 1 sh juv. (CV); surroundings of Semaforo south slope, 37°57'56"N 12°03'42"E, 430 m, 08.III.2019, 1 sh (CR); idem, 37°57'56"N 12°03'42"E, 430 m, 08.III.2019, 1 sh (CR); Pizzo Madonnuzza, north slopes, 37°59'19.6"N 12°03'26"E, 225 m, 09.III.2019, 1 sh juv. (CV); Case Romane, 37°58'14"N 12°03'51"E, 230/240

Figure 5. *Acicula hierae* n. sp., holotype: Italy, Sicily, Marettimo Island, Punta Campana, H: 4 mm, with soft part.
Figure 6. *Acicula hierae* n. sp., paratype: Italy, Sicily, Marettimo Island, Carcaredda, H: 3.7 mm, SEM photo.

m, 09.III.2019, 2 sh (CMS 5220/2); Carcaredda, 37°57'16"N 12°04'30"E, 200 m, 10.III.2019, 3 sh (CR) (Fig. 6); Punta Campana, eastern slopes, 37°58'22"N 12°03'27"E, 490–520 m, 25.VIII.2020, 1 sh, legit R. Viviano (CV); Punta Campana, eastern slopes, base of a rocky wall, 37°58'21"N 12°03'25"E, 540 m, 25.VIII.2020, 3 sh, legit R. Viviano (CV).

DIAGNOSIS. Conic-subcylindrical shell, with obtuse apex, well developed angular tooth and parietal callus; the upper part of the peristome forms a short canal with the angular tooth. External peristomal varix raised, rounded, delimited anteriorly and posteriorly by simple line, in lateral view narrower in the upper part and wider in the lower one; 5–5 ½ whorls, last whorl 47–50% of shell height; 12–16 spaced radial grooves on penultimate whorl.

DESCRIPTION OF HOLOTYPE. Shell (Fig. 5) dextral, small, little elongate, conic-subcylindrical, with obtuse apex, glassy and transparent, light brown in color with a thin subsutural darker brown band. Spire consisting of 5 ½ slightly convex whorls. Last whorl 50% of shell height, sutures shallow. External surface of teleoconch smooth, with irregularly spaced radial grooves, 14 in penultimate whorl. Aperture oval-pyriform; thickened peristome, rounded on the columellar side; upper and lower vertices joined by thick parietal callus; well developed angular tooth; the upper part of the peristome forms a short canal with the angular tooth. External peristomal varix raised, rounded, and delimited anteriorly and posteriorly by simple line, in lateral view it is narrower in the upper part, wider in the lower one; Umbilicus closed. H: 4 mm, D: 1.25 mm, LWH: 1.9 mm.

Operculum H: 0.8 mm, D: 0.6 mm; drop-shaped, very thin, transparent, sand-yellow in colour, paucispiral with semi-central nucleus; outer face with about 9 growth lines; smooth inner face, with muscle attachment area comma-shaped.

Body whitish; black round pupils and little black bacillus-shaped spots at the base of the tentacles; internal organs withish.

VARIABILITY. The paratypes do not show substantial morphological differences with the holotype: H: 3.3–4 mm, mean 3.8 mm, D: 1.2–1.3 mm; spire of 5–5 ½ whorls; last whorl 47–50% of shell height, 13–19 radial grooves in penultimate whorl.

ETYMOLOGY. The new species is named after "Hiera Nesos" (= Sacred Island) ancient name, first Greek and then Latin, of Marettimo island.

DISTRIBUTION AND BIOLOGY. *Acicula hierae* n. sp. is until now known only from the type locality Marettimo Island (Fig. 1).

It occurs in natural habitats with Mediterranean maquis (mainly *Salvia rosmarinus* Spenn., *Erica multiflora* L., *Cistus* spp, *Euphorbia dendroides* L., 1753, *Pistacia lentiscus* L.) and oaks woods.

It lives in the crevices of dolomitic rocks, under stones and underground environments.

STATUS AND CONSERVATION. The restricted distribution makes *A. hierae* n. sp. "Vulnerable", according to the Categories and Criteria of the IUCN Red List of Threatened Species (IUCN, 2020).

COMPARATIVE NOTES. *Acicula hierae* n. sp. differs from the geographically closest *Acicula* species, *A. benoiti* from Sicily, in its lower height (3.3–4 mm for *A. hierae* n. sp., 3–4.7 mm for *A. benoiti*) lower height-to-width ratio (2.9 for *A. hierae* n. sp., 3.3 for *A. benoiti*), the highest last whorl (47–50% of the total height in *A. hierae* n. sp., 22–27% in *A. benoiti*), lower number of whorls (5–5 ½ in *A. hierae* n. sp., 5 ½–7 ½ in *A. benoiti*), canal between the angular tooth and the upper edge of the peristome, more developed in *A. hierae* n. sp.; external peristomal varix more raised, and delimited anteriorly and posteriorly, while in *A. benoiti* more like a swelling, without clear demarcation in the basal part; in lateral view the esternal peristoma varix is wider in the lower part in *A. hierae* n. sp. while in *A. benoiti* the same width troughout its lenght is shown.

From the North African *Acicula* species, *A. hierae* n. sp. is well differentiated from *A. letourneuxi* (Bourguignat, 1864) (see Boeters et al., 1989: 59, Fig. 59) while it is more similar to *A. algerensis* Gittenberger et Boeters, 1977 (Algeria and Morocco) and to *A. lallemanti* (Bourguignat, 1864) (Algeria, Tunisia, Libya). The new species differs from *A. algerensis* and *A. lallemanti* for the more developed angular tooth and external peristomial varix, and for the greater height of the last whorl. In addition, it differs from *A. lallemanti* in a more compact shape of the shell and the lower number of furrows on the penultimate whorl (24–34 in *A. lallemanti*) (see also Gittenberger & Boeters, 1977b; Boeters et al., 1989).
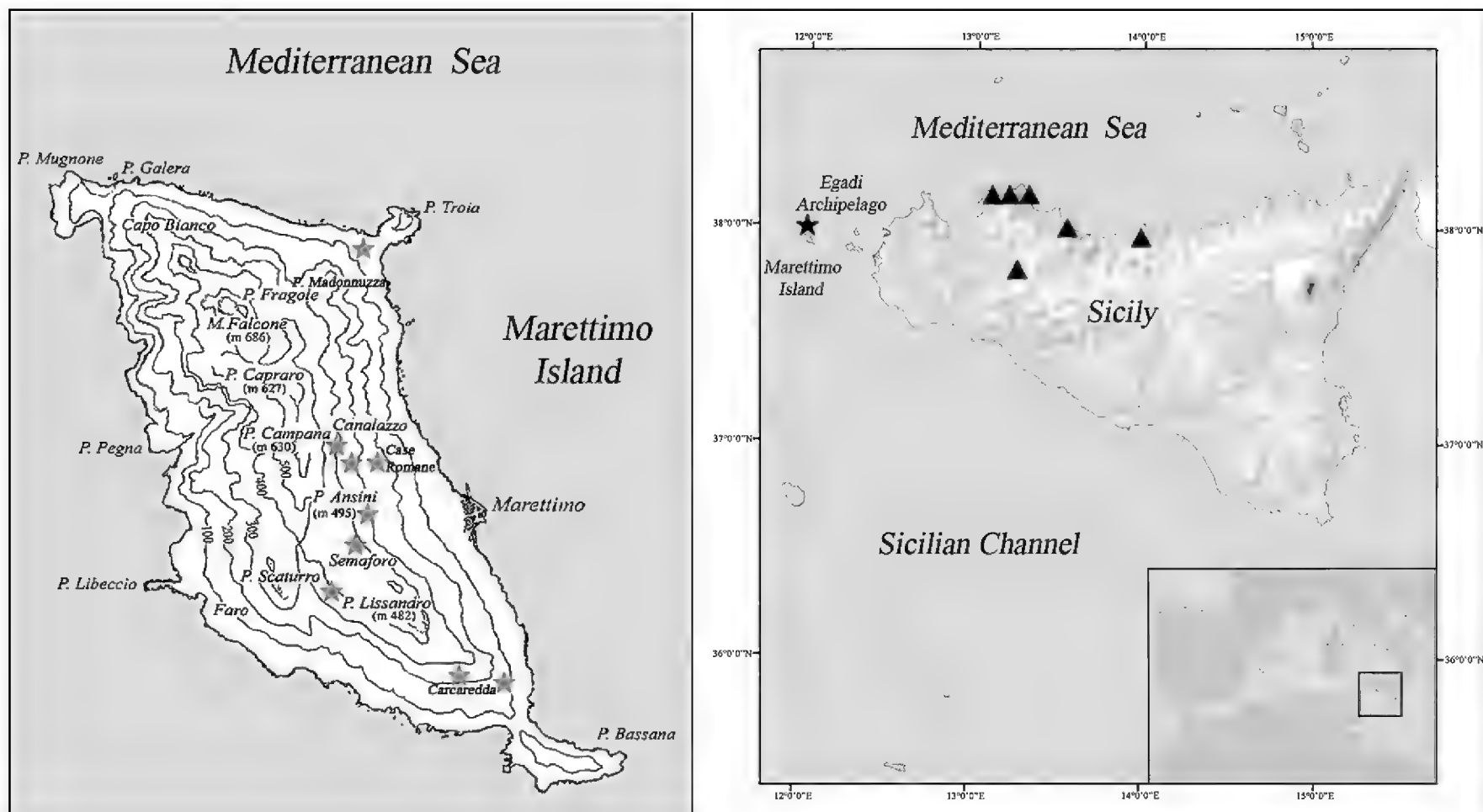
Figure 7. Distribution map of *Acicula benoiti* (triangles) and *A. hierae* n. sp. (stars) according to the examined material.

Acicula szigethyannae Subai, 1977 (Italy: from Liguria to Calabria) differs mainly from *A. hierae* n. sp. for the subquadrangular aperture, less developed angular tooth, external peristomal varix flat and crossed by furrows, the greater number of furrows (19–27) on the penultimate whorl (Subai, 1977; Eikenboom, 1998; Feher, 2013).

Fiorentino et al. (2004) reports *A. benoiti* for the Marettimo island, but in our samples only *A. hierae* n. sp. has been found.

Among the uncertain taxa described for Marettimo, the systematic position of *Caecilianella maretima* Benoit, 1882 remains to be clarified. The Sicilian author (Benoit, 1882) provides a very simple description, however, based on morphological affinities, he adds this new species in the *Cecilioides-Hohenwarthiana* species group. In the same work the Sicilian *Acicula* species is attributed to the genus *Acme* (Benoit, 1882 sub *A. lineata = A. benoiti*). Alzona (1971) reports "*maretima* Benoit, 1882" among the dubious species belonging to the genus *Hohenwarthiana* Bourguignat, 1864.

CONCLUSIONS

Acicula hierae n. sp. is added to the endemic land molluscs of Marettimo, that include five other species: *Siciliaria scarificata* (L. Pfeiffer, 1856); *Oxychilus (Hyalofusca) denatale* (L. Pfeiffer, 1856); *Schileykiella bodoni* Cianfanelli, Manganelli et Giusti, 2004; *Cernuella (Xeroamanda) depressior* (Benoit, 1859); *Marmorana insularis* (Benoit, 1857).

ACKNOWLEDGEMENTS

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A new species of the *Alvania pagodula* group (Monterosato, 1890) from the Pliocene of Italy: *A. agathae* n. sp. (Gastropoda Rissoidae)

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ABSTRACT   *Alvania agathae* n.sp. is described from the Pliocene of Italy. The new species is based on 17 fossil shells found in Pliocene layers of Sicily and Tuscany and belongs to the genus *Alvania* (Risso, 1826). *Alvania agathae* n.sp. is relatively close to *A. spinosa* (Monterosato, 1890), grouped within *Alcidiella* Cossmann, 1921, usually treated as a subgenus or synonym of *Alvania*. The description and the comparison with other Mediterranean similar fossil and living species are here reported. The possible phylogeny of the new, as well as related species included *A. spinosa*, is also discussed.

KEY WORDS   Rissooidea; *Alvania*; Pliocene; new species; Buccheri; Orciano Pisano.

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INTRODUCTION

Rissoidae, with related genera, is one of the most diverse families of marine molluscs in the world. In particular, species of the genus *Alvania* (Risso, 1826) inhabit a large variety of different environments, from shallow to bathyal depth, in the Mediterranean Sea, the Atlantic, the Indo-Pacific and the temperate Australian coasts (Ponder 1985). Many authors contributed to the study of the family Rissoidae, describing many new taxa and expanding our knowledge on the taxonomy of this fascinating group.

In this paper, we describe *Alvania agathae* n.sp., a new remarkable species of the genus *Alvania*, based on fossil shells found in Pliocene layers of Sicily and Tuscany. This new species belongs to a group of species once grouped under the subgeneric name *Alcidiella* Cossmann, 1921, nowadays accepted as a synonym of *Alvania*, which is poorly studied and on which there are gaps in knowledge on the difference between species. Our findings allow us to discuss the presence of *Alcidiella* in the Mediterranean Plio-Pleistocene and to hypothesise a possible phylogeny between the fossil and living species closest to *A. agathae* n. sp.

MATERIAL AND METHODS

For the present paper, 30 kilos of sediments were collected from a sandy layer with *Persistrombus coronatus* (Defrance, 1827) cropping out in contrada Pirazzo, 500 m north of Masseria Pirazzo (37°9'28.63"N; 14°52'25.03"E; 608 m a.s.l.) along

the east side of Monte Costerotte (851 m) at about 4 km from North-East of Buccheri, Siracusa, Italy. Further 50 kilos of sediments were collected from a sandy layer with *Euspira helicina helicina* (Brocchi, 1814) from excavation sites used for the construction of houses near the town of Orciano Pisano, Pisa, Italy.

Sediments were routinely washed, dried and sieved. Then, shell specimens were picked out from the > 10 mm fraction. The shell specimens were examined, measured and photographed under a stereomicroscope. In particular, the holotype and the Paratype 1 were also examined uncoated under a Zeiss LEO 1455 VP SEM microscope in Low Vacuum modality, to investigate its micromorphology. The repository number of the holotypes and the paratypes are given in the systematic descriptions.

ACRONYMS. Museo Civico di Storia Naturale di Comiso, Ragusa, Italy (MCSN); Massimo Cresti malacological collection, San Casciano in Val di Pesa, Firenze, Italy (MCC); Danilo Scuderi malacological collection, Belpasso, Italy (DSC), Alberto Villari malacological collection, Messina, Italy (AVC); Attilio Pagli malacological collection, Empoli, Italy (APC).

RESULTS

Systematic Palaeontology

Phylum MOLLUSCA Cuvier, 1797
Classis GASTROPODA Cuvier, 1795
Subclassis CAENOGASTROPODA Cox, 1960
Ordo LITTORINIMORPHA Golikov et Staroboga-
    tov, 1975
Superfamilia RISSOOIDEA Gray, 1847
Familia TROCHIDAE Rafinesque, 1815
Genus *Alvania* Risso, 1826
Type species: *Alvania europea* Risso 1826 = *Turbo cimex* Linnaeus, 1758

Alvania agathae n. sp.
http://zoobank.org:act:44508215-7C04-49FC-
ADA3-BBB675C69DEF

TYPE LOCALITY. Pliocene of contrada Pirazzo, Buccheri, sandy layers with *Persistrombus coronatus* (Defrance, 1827), Siracusa, South-East Sicily, Italy (details under stratigraphy and paleoenvironment below).

EXAMINED MATERIAL. Holotype (Fig. 1), contrada Pirazzo, Buccheri, Siracusa, Sicily, Italy, (MSNC 4723). Paratypes and specimens same data of the holotype: paratype B and C (Figs. 2, 3) and paratype A (Fig. 4) (MSNC 4724), paratype D (Fig. 5) and E (MCC) from Orciano Pisano, paratypes F-R, and 8 specimens (MSNC 4725); 2 specimens (DSC), 1 specimen (AVC).

DESCRIPTION OF THE HOLOTYPE. Shell conic, slender, slightly perforated, with a weak umbilical chink, sometimes absent and a pointed spire, slightly less high than the last whorl; height 3.2 mm, width 2.03 mm, H/W 1.5. Protoconch eroded (Fig. 1).

Just below the protoconch boundary, in the first tele-whorl, the median spiral keel could be detected. Teleoconch is constituted by 5 whorls, with weak sutures and a wide subsutural ramp. Sculpture is characterised by a marked spiral keel in the middle of the each whorl and axial ribs which form prominent spines at the intersection. The axial ribs and relative spines are thicker than the spiral keels, orthocline, 9 in number on the early tele-whorls, 11 on the body whorl. In the last whorl, which is about 1/3 of the total length of the shell, a single spiral keel with 11 spines is present. From the upper insertion of the external lip to the base, there is a second keel below which the axial ribs are not visible. In fact, the base of the shell below the second keel is completely smooth. From the penultimate to the first tele-whorl this keel is visible as a faint upper-sutural cord.

The aperture is ovate and slightly drop-shaped, with thin partially damaged peristome. The umbilicus is narrow.

VARIABILITY. Paratypes variation: shell height 3.1-3.5 mm, width 2.02-2.05, H/W 1.5-1.7 (Figs. 2, 3, 5). Protoconch (paratype A, Fig. 4) almost smooth, slender, conical, paucispiral, consisting of 1.5 regularly convex whorls with only thin and faint growth-lines; height 0.25mm, maximum diameter 0.27 mm; nucleus inflated and inrolled; diameter 0.1 mm; transition boundary from proto to teleoconch is marked (Figs. 8, 9, 12, 13).

Teleoconch with 5-5.5 whorls; axial ribs and relative spines 10-11 in number on the body whorl. In the last whorl 10-12 spines are present. A clear spiral micro-sculpture, detectable only at high magnifications, is present over all the shell surface but
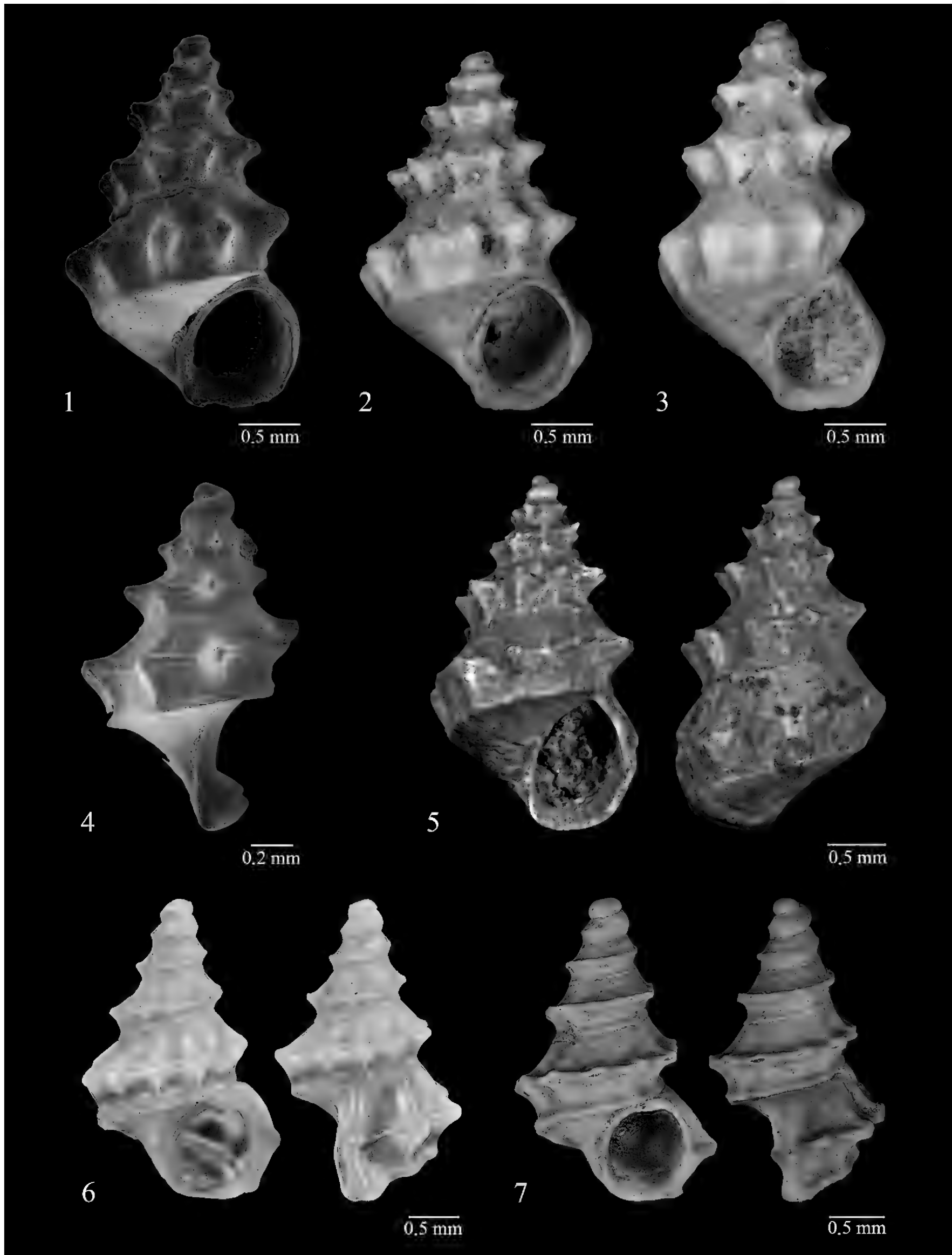
Figures 1–4. *Alvania agathae* n.sp., from type locality. Fig. 1: holotype, h: 3.3 mm. Fig. 2: paratype B, h: 3.1 mm. Fig. 3: paratype C, h: 3.5 mm. Fig. 4: paratype A, h: 1.63 mm. Figure 5. *Alvania agathae* n.sp., paratype D, from Orciano Pisano, h: 2.95 mm. Figure 6. *Alvania tessellata* Schwartz in Weinkauff, 1868, from Getares (Spain), beached in shell grit, h. 2.98 mm (APC). Figure 7. *Alvania alboranensis* Peñas et Rolán, 2006 , from Motril (Spain), -110 m in shell grit, h. 2.78 mm (APC).

better evident on the ab-sutural and ad-sutural zone of each whorl, where spiral lines are closer to each other (Fig. 14).

ETYMOLOGY. The specific name is after the Agata Conti (Catania, Italy), mother of the first author (A.R.).

STRATIGRAPHY AND PALEOENVIRONMENT. Buccheri (Siracusa, Sicily, Italy). Sediments were collected in contrada Pirazzo in lenticular sandy and calcarenitic layers, located in the area of Buccheri. They were characterised by the presence of Persistrombus coronatus (Defrance, 1827) and by a rich malacofauna from pliocenic shallow marine environments (Philippi, 1844; De Gregorio, 1882; Alemagna, 1920; Glibert, 1960; Glibert & Van de Poel, 1965; Grasso et al., 1979; Carbone et al, 1986; La Perna, 1999). Molluscan assemblages are characterized mainly by the *Posidonia* meadows (HP) and photophilic algae (AP) biocenosis (sensu Pérès & Picard, 1964): *Tricolia pullus* (Linnè, 1758), *Jujubinus* spp., *Clanculus* spp., *Persistrombus coronatus* (Defrance, 1827); fine-grained well sorted sands biocenonsis (SFBC): *Glycymeris* spp., *Spisula subtruncata* (Da Costa, 1778), *Neverita olla* (De Serres, 1829), *Chamelea gallina* (Linnaeus, 1758); superficial muddy sands in sheltered areas biocenosis (SVMC): *Loripes lacteus* (sensu Poli, 1791), *Bittium deshayesi* Cerulli-Irelli,1912; coastal detritic biocenosis (DC): *Tellina serrata* Brocchi, 1814, Erato spp. and muddy detritic bottom biocenosis (DE): *Timoclea ovata* (Pennant, 1777), *Plagiocardium papillosum* (Poli, 1795), *Bolma rugosa* (Linnaeus, 1767), *Calyptraea chinensis* (Linnaeus, 1758).

Orciano Pisano (Pisa, Tuscany, Italy). The material from Orciano Pisano was sampled in grayish clay with *Euspira helicina elicina* (Brocchi, 1814), *Ringicula buccinea* (Brocchi, 1814), *Pagodula echinata* (Kiener, 1839), probably belonging to the "facies Piacenziana" (Brunetti et al, 2017 and references therein; Brunetti & Cresti, 2018), characterized by shell assemblage from circalittoral and epibathial paleoenvironments.

REMARKS. *Alvania agathae* n. sp. belongs to the so-called *A. pagodula* group, including four different extant species Mediterranean species (WoRMS 2020): *A. alboranensis* Peñas et Rolán, 2006 (most probably a subfossil species), *A. pagodula* (Bucquoy, Dautzenberg et Dollfus, 1884), *A. spinosa* (Monterosato, 1890) and *A. tessellata* Schwartz in

Weinkauff, 1868. These were previously grouped within the subgeneric name *Alcidiella* (Van Aartsen, 1976). However, Ponder (1985) did not give any taxonomical significance to the latter, in spite of the common features shared between the four species (Van Aartsen, 1976, Palazzi, 1996). The Miocene fossil species *A. munita* Palazzi, 1996, included in *Alcidiella* by the author, shares some features with the *A. pagodula* group and partially with *A. agathae* n. sp., but is different on account of a greater number of spiral cords.

At the current state of knowledge, no other Tertiary fossil *Alvania* species are directly comparable with *A. agathae* n. sp.

Alvania spinosa is known as fossil record from Pleistocene layers of Gravitelli and Milazzo (Seguenza G., 1873–1874; Seguenza L., 1903; Greco & Lima, 1974); we found some further specimens from the inferior Pleistocene (Sicilian stage) of Augusta (Di Geronimo et al., 2000). Seguenza L. (1903) did not provide any description or draw of *Alcidia spinosa*, ex *A. angulata* (Seguenza G., 1874, not 1876)= *Alcidia spinosa* (Monterosato, 1890) and for this reason, it is not possible to correctly address this species to any other known *Alvania*. Furthermore, Seguenza Luigi's collection (originally owned by his father Giuseppe) got lost and partially destroyed during the earthquake of 1908 (Bertolaso & Palazzi, 2000). However, Monterosato (1890) synonymised "R. angulata *(not Eichwald, 1830) Seguenza mss.*" as *Alcidia spinosa*; although the reason for such choice is unknown.

Tringali (2001), based on actual specimens collected in Mediterranean shores of Morocco, claims that *A. spinosa* (Monterosato, 1890) sensu Pallary (1902, 1920) is a morphotype of *A. tessellata*, considering the great variability shown by the latter species. Such assumption was recently accepted by Gofas et al. (2011), although it was not reported in WoRMS (2020). *Alvania spinosa* s.s. is characterized by a single median keel bearing spines, while *A. tessellata* presents a second less developed keel with spines, located halfway between the median region and the suture. However, Tringali's hypothesis was not based on the direct comparison between his samples and the holotype of *A. spinosa* (as reported in his study, the author was not able to find it in Monterosato's collection housed in the zoological museum in Rome) or specimens from Thyrrenian Sea. Moreover, the figure (42: 23 C) of
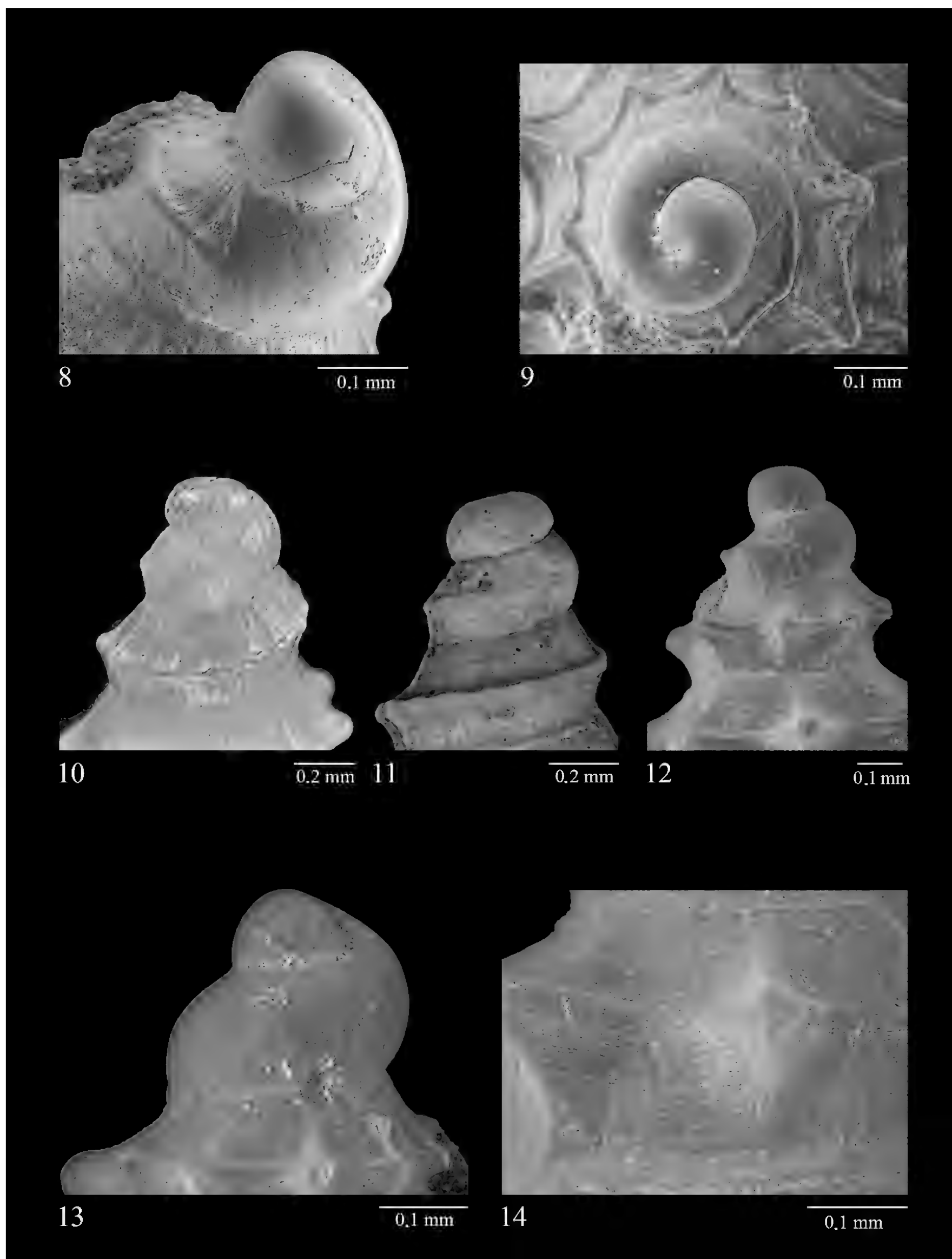
Figures 8, 9, 12, 13. *Alvania agathae* n.sp, paratype A from type locality, details of apex. Figure 10. *Alvania tessellata* Schwartz in Weinkauff, 1868, from Getares, (Spain) beached: detail of apex (APC). Figure 11. *Alvania alboranensis* Peñas et Rolán, 2006, from Motril (Spain) -110 m in shell grit: detail of apex (APC). Figure 14. micro-sculpture of *Alvania agathae* n.sp, paratype A.

the specimen of *A. spinosa* (Institut Royal des Sciences Naturelles de Belgique, ex Monterosato) reported in Ponder (1985), does not match with the original description of *A. spinosa* and rather looks like a specimen of *A. tessellata* s.s.

According to the recent literature (Giannuzzi-Savelli et al., 1996; Cossignani, 2011; Scaperrotta et al., 2012), *A. spinosa* is distributed only in the Alboràn Sea.

Probably, specimens referable to *A. spinosa* s.s. belong to the suprapleistocenic thanatocenoses from the deep sea in the low Tyrrhenian Sea.

In light of this, *A. agathae* n. sp. will be compared mainly with *A. tessellata* and its morphotype *A. spinosa* sensu auctores.

Alvania agathae n.sp. differs from *A. spinosa* sensu auctores in having a smoother apex, a bigger size, a different height/last whorl ratio, a wider and completely smooth base, a simple and thin peristome lacking of spines, and a more prominent teleoconch with pointed spines.

Alvania agathae n.sp. differs from *A. tessellata* s.s. in having a single median keel (Fig. 6); the species is more similar to *A. alboranensis* (Fig. 7), which also have a hint of axial sculpture. However, *A. agathae* n. sp. differs from *A. alboranensis* in its keel, located in the last whorl starting from the superior margin of the peristome; furthermore, the keel in *A. agathae* n. sp. is smooth and sharper. Ultimately, *A. agathae* n.sp. has a more or less convex basis instead of canaliculated, being this latter character characteristic of *A. alboranensis* s.s.

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A new species of *Neopiciella* Sama, 1988 (Coleoptera Cerambycidae) from western Sicily

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ABSTRACT   A new species of the genus *Neopiciella* Sama, 1988 (Coleoptera Cerambycidae) from Sicily is described. *Neopiciella stefaniae* n. sp. is compared with the other species of this genus: *N. sicula* (Ganglbauer, 1885) from Sicily and *N. kabyliana* (Pic, 1896) from Algeria and Tunisia. Further information on the taxonomy, biology and geographical distribution of this small group of Beetles, and on *N. stefaniae* n. sp. in particular, are provided.

KEY WORDS   Coleoptera, Cerambycidae; *Neopiciella*; new species; Sicily; North Africa.

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INTRODUCTION

The genus *Neopiciella* Sama, 1988 (Coleoptera Cerambycidae) has been introduced by Sama (1988) as a new name for *Piciella* Villiers, 1946 "*(nomen nudum) nec Borchmann, 1936*" with the following morphological characters: "*pronoto più lungo che largo, munito di una sporgenza arrotondata ai lati poco avanti la metà, senza evidente solco trasversale prebasale. Antenne robuste con articoli terminali ingrossati, 4° articolo più corto del 3°, 6° non più corto del 3°. Elitre ristrette verso l'apice, qui arrotondate. 2° articolo dei tarsi posteriori non più lungo del 3°*" [pronotum longer than wide, with a rounded projection on the sides just ahead of the middle, without evident transverse prebasal groove. Antennae robust with thickened end segments, 4th segment shorter than 3rd, 6th segment no shorter than 3rd. Elytra narrowed towards the apex, rounded here. 2nd segment of the posterior tarsus no longer than the 3rd]. The type species of the genus is *Leptura sicula* Ganglbauer, 1885 designed by Sama for *Neopiciella*. Villiers (1946) described *Piciella* without original designation.

Currently, this genus includes two species: *N. sicula* (Ganglbauer, 1885) from Sicily with type locality "Sicilie" (Ganglabauer, 1885) and *N. kabyliana* (Pic, 1896) (= *suberis* Chobaut, 1900) from Algeria and Tunisia with type locality "*Algérie: forêt de Yakouren*" (Pic, 1896).

Particularly, *N. sicula* is an uncommon species, cited for a few Sicilian locations, although it is widespread on the island along the northern mountain ridge from the Peloritani to the Sicani Mountains and around Palermo (Ragusa, 1924; Vitale, 1936; Sama & Schurmann, 1980; Sparacio, 1999; Baviera & Sparacio, 2002; Sabella & Sparacio, 2004; Sama, 2005; Rapuzzi & Sama, 2006; Bellavista et al., 2008; La Mantia et al., 2010; Rapuzzi, 2010; Baviera et al., 2017).

Neopiciella sicula is also reported as Critically Endangered (CR) in the Red List of Italian Saproxylic Beetles (Rapuzzi et al., 2014; Carpaneto et al., 2015).

In recent years, other new localities have been surveyed for *N. sicula* up to Monte Inici (Trapani) (Baviera et al., 2017). Further researches carried out on Sicilian xylophagous beetles highlighted the

presence of this Cerambycidae also in other wood formations of the Trapani area. The study of these western populations has allowed us to highlight substantial morphological differences with the other known populations of *Neopiciella* and to describe a new species below.

ACRONYMS. CGS = Gianfranco Sama, Cesena, Italy, private collection. CIS = Ignazio Sparacio, Palermo, Italy, private collection. CMC = Michele Carraretto, Morgano, Treviso, Italy, private collection. CMB = Michele Bellavista, Palermo, Italy, private collection. CPR = Pierpaolo Rapuzzi, Cialla di Prepotto, Udine, Italy, private collection. CSN = Stefano Nappini, Castiglione della Pescaia, Grosseto, Italy, private collection. NHMW = Naturhistorisches Museum Wien, Wien, Austria.

RESULTS

Systematics

Ordo COLEOPTERA Linnaeus, 1758
Familia CERAMBYCIDAE Latreille, 1802
Subfamilia LEPTURINAE Latreille, 1802
Tribus LEPTURINI Latreille, 1802
Genus *Neopiciella* Sama, 1988

Neopiciella stefaniae n. sp.   (Figs. 1, 2, 5, 6)
htpp://zoobank.org:act:BE3F2957-46DF-45E9-9C0C-AB66A3B81242

TYPE MATERIAL. Holotypus male: Italy, Sicily, Trapani, Castellammare del Golfo, Monte Inici, 12.VI.2009 (CMB). Paratypes: same data of the holotype, 25.VI.2000, 1 ex (CMB); idem, 12.VI.2009, 5 ex (CMB); idem, 21.VII.2010, 1 ex (CMB); idem, 21.VI.2020, 5 males and 2 females (CIS); Bosco Scorace, 21.VI.2020, 1 male (CIS); Monte Sparagio, 21.VI.2020, 1 male and 2 females (CIS); Monte Sparagio, 28.VI.2020, I. Sparacio legit, 1 male (CPR); Monte Inici (TP), 10.VI.2009, I. Sparacio legit, 2 males and 1 female (CPR); idem, 1 male and 1 female, 21.VI.2020 (CPR).

OTHER EXAMINED MATERIAL. *Neopicella sicula*. Holotype (Fig. 1): the type series is made by a single specimen (female) in discreet conditions (it is missing the right hind tibia and the left tarsi of the hind leg, the right elytron shows a big hole due to an old pin), preserved in the Ganglbauer collection (NHMW). The specimen has the following labels: Sicilia [white label, handwritten by Ganglbauer] / Leptura si=/cula Gglb. Typ. [white label, handwritten by Ganglbauer] / TYPE [red label, printed] / sicula/Sicil. Ganglb. [white label handwritten by Ganglbauer].

Italy, Sicily, Palermo province, Piana degli Albanesi, 24.VI.1995, 1 male (CIS); Madonie Mountains: Pomieri, 17.VI.2008, 1 female (CIS); idem, 28.VI.2008 2 males and 1 female (CIS); idem, 6.VI.2009, 1 female (CIS); Bosco Ficuzza, Alpe Cucco, 20.VI.2009, 1 male (CIS); Bosco Ficuzza, Bivio Lupo, 20.VII.2009, 1 male (CIS); Palermo, 3.V.2001, 1 female (CIS); Palermo, 8.V.2020, 1 female (CIS); Bosco della Ficuzza, Fanuso, 25.VI. 2009, 1 male (CMB); idem, Alpe Cucco, 20.VII.2009 (CMB); Messina province, Portella Obolo, 16.VI–7.VII.2006, P. Rapuzzi & G. Sama leg., 4 males and 3 females (CPR); Messina province, Mt. Soro, 16.VI–7.VII.2006, P. Rapuzzi & G. Sama lgt., 4 males + 1 female (CPR); Palermo province, Gibilmanna, 15.VI–6.VII.2006, P. Rapuzzi & G. Sama leg., 1 female (CPR); Madonie Mountains, 1430 m., Piano Battaglia, 15.VI-6.VII.2006, P. Rapuzzi & G. Sama leg. (CPR); Madonie Mountains, Vallone Pomeri, 1300–1500 m., 14.VI–5/6.VII.2006, P. Rapuzzi & G. Sama leg., 11 males + 7 females (CPR); Madonie Mountains, Piano Zucchi, 1300 m., 13.VI–8.VII.2006, P. Rapuzzi & G. Sama leg., 4 males (CPR); idem, 6.VII.1991, P. Rapuzzi leg., 1 female (CPR); Madonie Mountains, Castelbuono, Rifugio Crispi, m.1300,4.VI–7.VII.2019, M. Malmusi lgt. (CSN); 1 female; Madonie Mountains, Piano Zucchi, 1200-1300 m., 2.VI.2019, ex larva *Fagus* sp., S. Nappini leg., 1 male and 1 female (CSN). Madonie Mountains, Gibilmanna, loc. Piano delle Fate, 795 m., 16.VI-6.VII.2019, 1 male, M. Trentini leg. (CMC); Madonie Mountains, Piano Battaglia, 1639 m., 3 males, 37°52'46"N 14°30'18"E, 21.VI–19.VII.2020 (CPR); Nebrodi (Messina province), N of Capizzi, 1419 m., 1 male, 37°53'33"N 14°01'25"E, 20.VI–18.VII.2020 (CPR); Malabotta Forest (Messina province), 1188 m., 37°58'19"N 15°03'19"E, 18.VI–16.VII.2020 1 male (CPR); Malabotta Forest (Messina province), 1224 m., 37°58'22"N 15°03'06"E, 18.VI–16.VII.2020, 1 male (CPR); Madonie Mountains, Baita dei Faggi 1336 m., 37°52'39"N 14°00'32"E, 21.VI–

19.VII.2020, 1 female (CPR); Malabotta Forest (Messina province) 37°58'29"N 15°03'04"E, 1218 m., 18.VI–16.VII.'20, 1 male (CPR); Nebrodi (Messina province), Portella Obolo 1502 m., 37°54'00"N 14°30'07"E, 20.VI-18.VII.2020, 2 males (CPR).

Neopiciella kabyliana (Figs. 4, 5). Tunisia, Jendoouba, Aïn Draham, 1000 m., ex larva *Quercus suber*, sfarf. 3.VI.1991 G. Sama legit, 2 females (CGS); Algeria, Tizi Ouzou, Parc Nat. d'Akfadou, 1000–1400 m., ex larva *Quercus mirbecki*, 9.V.1987, G. Sama legit, 2 females (CGS).

DESCRIPTION OF HOLOTYPE. Length 1.01 mm. Body elongated, covered by fine and erect yellow setae denser on the pronotum and elytra sides. Head, pronotum, antennae, legs, and underside black; elytrae yellow with sides and apex blackish. Head elongated, feebly inclined forwards, irregularly punctured with a thin and deep median furrow. Antennal tubercles rounded and well separated.

Eyes globose, finely facets, emarginated at upper side, widely separated from the basis of mandibles. Antennae long and robust with thickened end segments, finely pubescent, inserted between the eyes and surpassing the elytral apex beyond almost all of the last segment. Scape densely punctured, fourth segment one third shorter than third, last segment slightly longer than the penultimate (ratio 1.16). Mandibles rather narrow and short, acute apically. Maxillary palpi with apical articles elongated, slightly wider in the basal third, truncated apically.

Pronotum slightly longer than wide, convex at the middle, narrowed forward and wider at the base with protruding posterior angles, sides with a rounded projection just ahead of the middle, with a long smooth median line from the base to the middle. Punctures wide, deep and well-spaced on the middle, smaller at front margin, juxtaposed and confused at the base.

Elytra less than three times longer than broad together, basis distinctly wider than pronotum, sides subparallel, narrower towards the apex, depressed along the entire side of whole length of the suture, sutures finely reboarded, surface covered by strong,
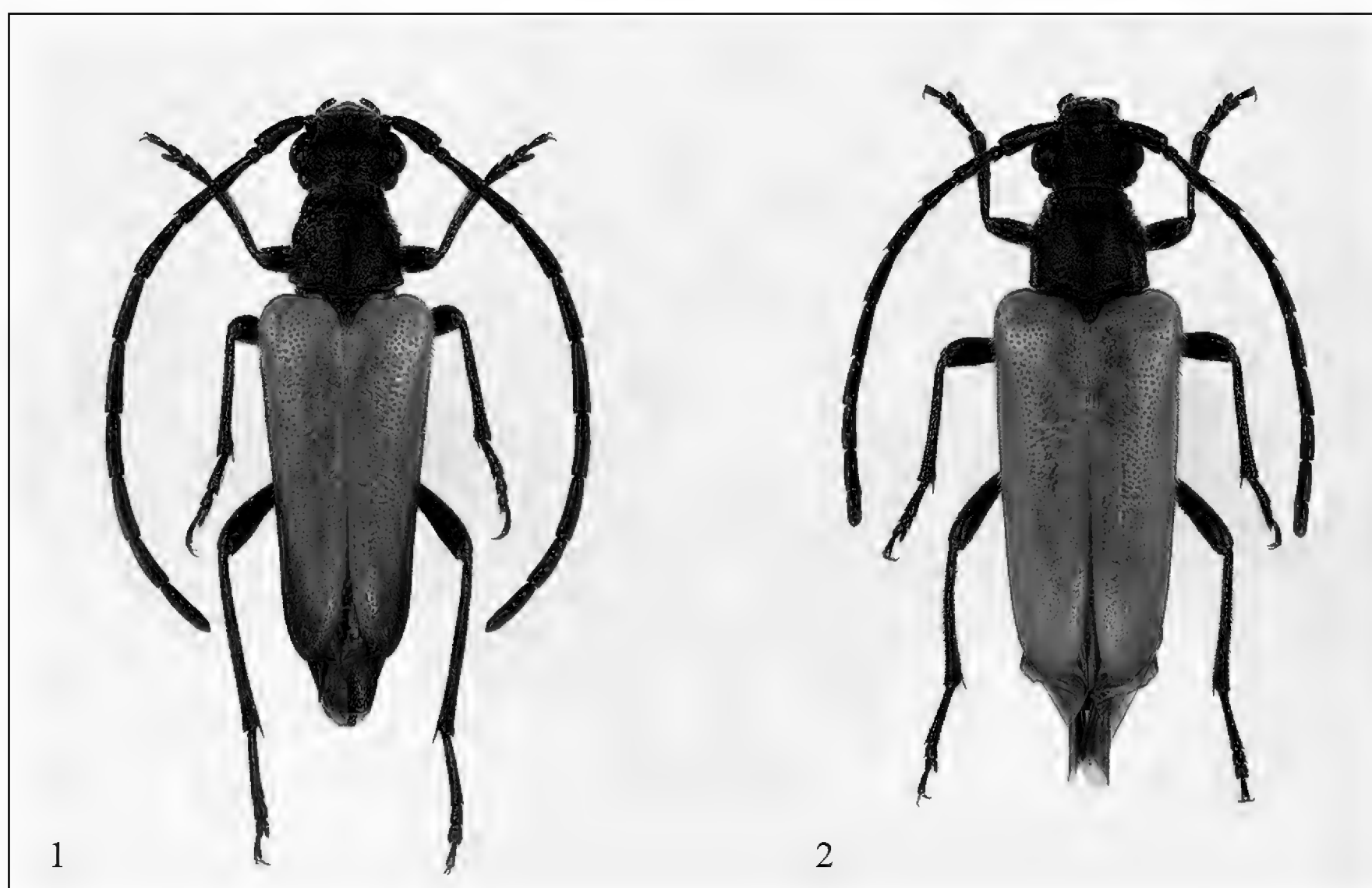
Figure 1. *Neopiciella stefaniae* n. sp., holotype male, Italy, Sicily, Trapani, Castellammare del Golfo, Monte Inici, 12.VI.2009. Figure 2. *Neopiciella stefaniae* n. sp., paratype female, same data of holotype.

large, irregular and dense punctures. Epipleurae well visible behind the humeri until the apex. Legs long with surface very finely punctured. Femora slightly curves, tibiae linear, Tarsi long, 2nd article shorter than the 3rd; onychium slightly toothed at the base.

Underside: prosternum with large and very superficial points, prosternum apophysis with enlarged apex and subrettilinear border; metasternum with thin median furrow, densely and finely punctured and with anterior apophysis broad and hollow in the center; sternites hollowed in the center with very small and very sparse points.

VARIABILITY. The male paratypes have no substantial morphological differences with the holotype described. The length is 0.95–1.05 mm, the blackish color of the elytra is sometimes more extensive. The females (Fig. 2) are larger (length 1.05–1.2 mm), with a more robust looking body, shorter antennae, prostrate apophysis hollowed in the center.

ETYMOLOGY. The species is named after Stefania Zaccolo from Cagliari (Sardinia, Italy), the first author's partner.

DISTRIBUTION AND BIOLOGY. *Neopiciella stefaniae* n. sp. is known, at the moment, only of the typical localities, in the westernmost part of Sicily. This new species seems to replace the similar *N. sicula* more widely diffused in the central-eastern part of the island, along the northern mountain ridge from the Peloritani to the Sicani Mountains and around Palermo (see Figs, 5, 6).

Neopiciella kabyliana is endemic from North Africa (Fig. 5): Algeria (Forêt de la Yakouren, La Calle, Djebel Edough, Guelma) and Tunisia (El Feidja) (see Villiers, 1946).

The biology of *N. stefaniae* n. sp. is unknown. Very likely it is similar to the other species of the genus. The larvas of *Neopiciella* feed between alive and dead wood of the stump of branches of the hosts. In some way it is very similar to the biology of *Pedostrangalia* Sokolv, 1896. All the known specimens were collected using sugar traps hanged on trees of *Quercus ilex* L. and *Quercus suber* L. It is very likely that these plants are the host of the new species.

Neopiciella sicula develops in the larval stage of dead parts plants living as *Fagus sylvatica* and *Quer-*
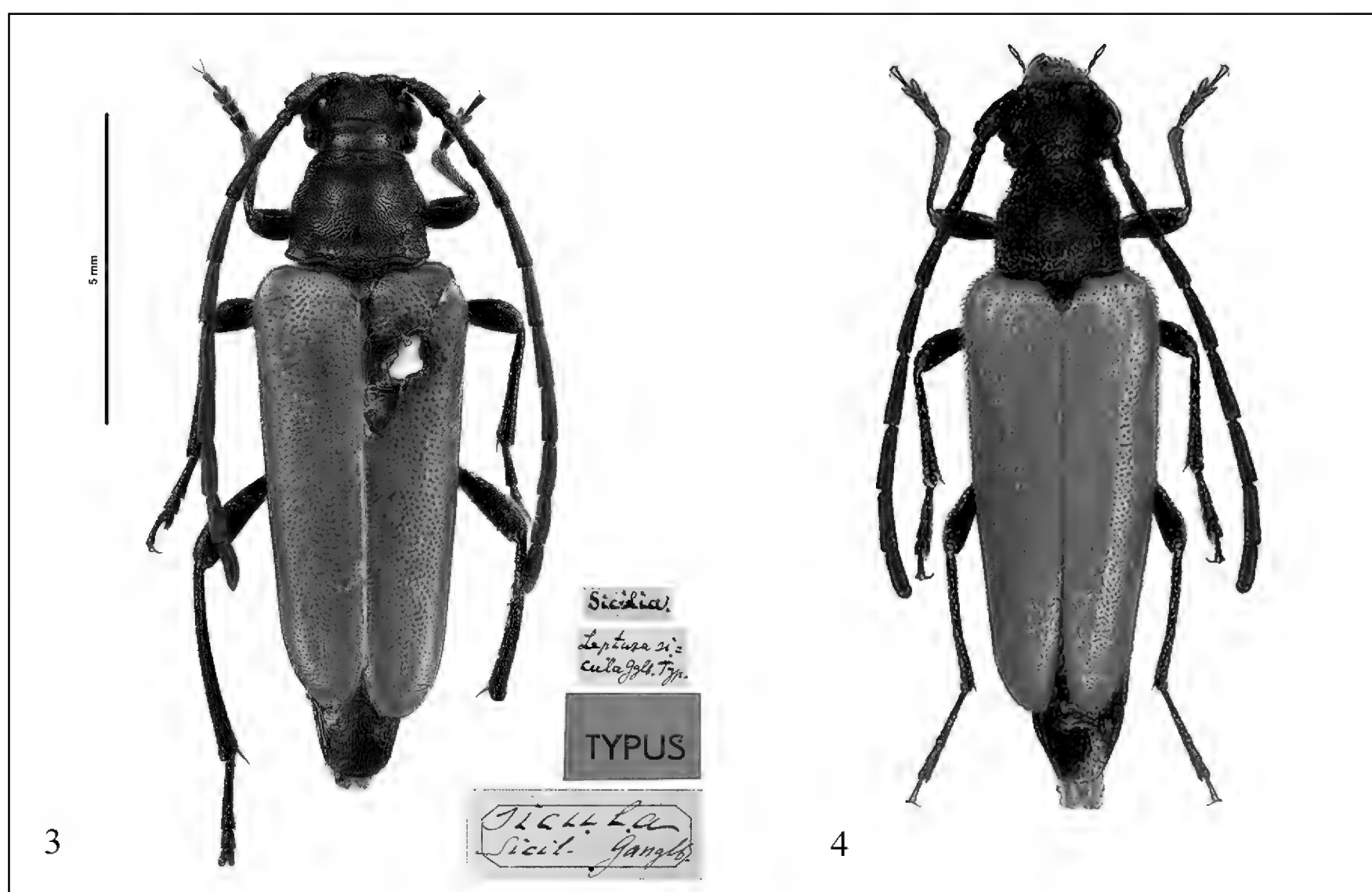
Figure 3. *Neopicella sicula*, holotype female. Figure 4. *Neopiciella kabyliana* female, Algeria, Tizi Ouzou, Parc Nat. d'Akfadou, 1000–1400 m.
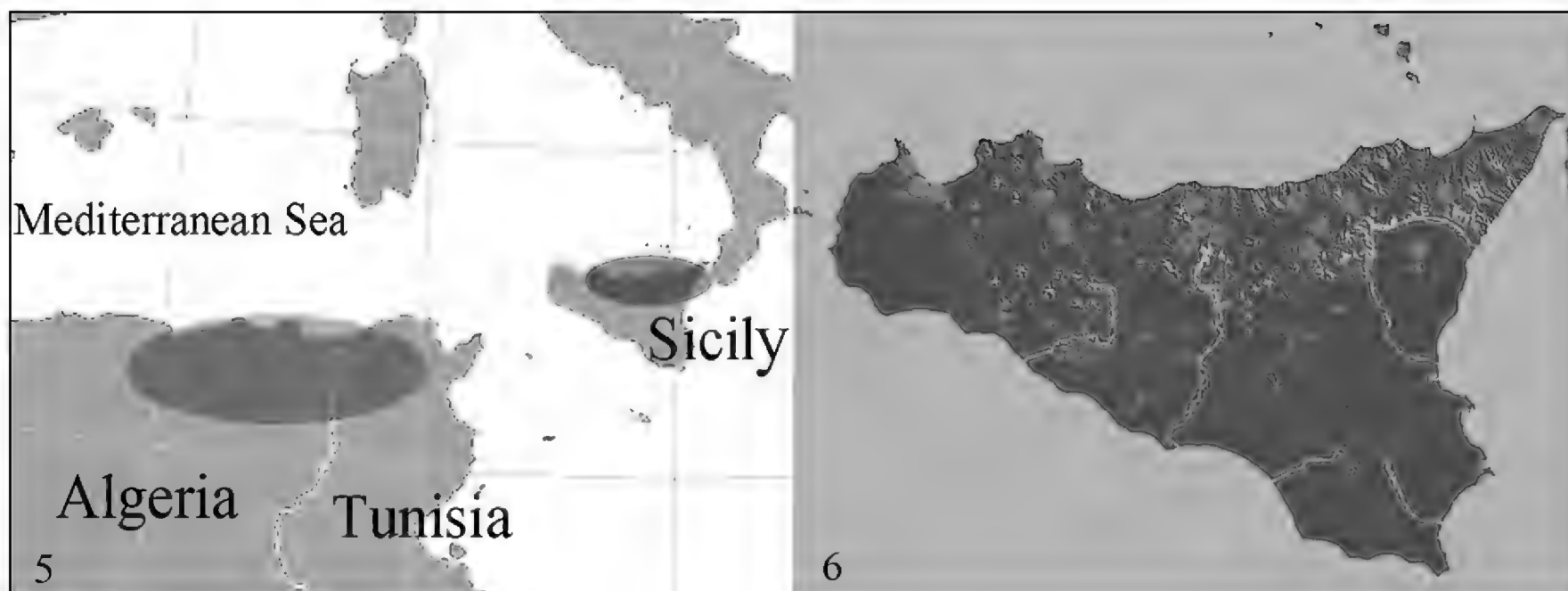
Figure 5. Maps of the genus *Neopiciella*: green = *N. sicula*, red = *N. stefaniae* n. sp., blue = *N. kabyliana*.
Figure 6. Maps of *N. sicula* and *N. stefaniae* n. sp. in Sicily.

cus (Sama & Schurmann, 1982) *Acer campestre, A. pseudoplanatus* (Sama, 1988). *Neopiciella kabyliana* was found on *Quercus suber* L. (Villiers, 1946).

The rarity of these *Neopiciella* species, and most likely also of *N. stefaniae* n. sp., is due to strictly arboreal habits of this beetles, rarely found on flowers or bushes.

REMARKS. *Neopiciella stefaniae* n. sp. is immediately distinguishable by the punctuation of the pronotum and, above all, of the elytra stronger, larger and denser punctures. In *N. sicula* and *N. kabyliana* this punctuation is smaller and more distant. The females of the new species show a shorter and stouter elytra, longer and parallel in the other known species.

Neopiciella stefaniae n. sp. differs from *N. sicula* for the last segment slightly longer than the penultimate (in males the ratio is 1.16 in *N. stefaniae* n. sp., 1.25 in *N. sicula*), the punctures of the underside smaller and more spaced, especially on the sternites, the prosternum apophysis of the males with subrettilinear apex (concave in *N. sicula*).

There are no significant differences between the aedeagus and tegmen of the new species compared with *N. sicula*. The apex is little more acuminate and the tegmen is little shorter in the new species. The 8th tergite of *N. stefaniae* n. sp. is evidently emarginate at the apex and about truncate in *N. sicula*.

It differs from *N. kabyliana* by the median shining line on the disk of the pronotum that is only on the basal half (this character is similar in *N. sicula*). The new species shows the sides of pronotum sinuate, similar with *N. kabyliana*, in *N. sicula* pronotum is smaller with the lateral sides more or less straight, with very small prominences on the middle. The punctuation is really deeper than both the other species of the genus. Tegmen of the new species is a little stouter than in *N. sicula*. Aedeagus is similar in both the species. We haven't seen any male of *N. kabyliana*.

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Overview of the arthropod fauna in the extreme southeastern Algeria: species richness in Tassili N'Ajjer National Park (Djanet, Algeria)

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ABSTRACT   The present study concerns arthropod populations in two different environments, cultivated and natural regions of Djanet. We conducted qualitative and quantitative surveys over two seasons (summer and winter). Three sampling methods were used: pitfall traps, sweep nets, and yellow pan traps. Overall, 4480 individual arthropods were captured representing 191 species, 4 classes, 21 orders and 106 families, distributed across four study sites, namely: Lokmane and El Mihane stations for the cultivated environment and Teghargharte and Iffoutten stations for the natural environment. A total of 112 species of arthropod species were captured by pitfall traps, with Hymenoptera accounting for 69% of the species. Sweep netting generated 65 species of arthropods, with Orthoptera accounting for 36% of the captures. Yellow pan traps led to the capture of 82 species of arthropods, with dipterans most attracted by this type of trap with a rate of 38%. The 17 species of Orthoptera that we captured in the region of Djanet belonged to 7 families and 2 orders, in which the species *Tridactylus variegatus* was the most abundant.

KEY WORDS   Arthropod species richness; Djanet, Cultivated and natural environment; traps.

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INTRODUCTION

The Tassili region of Algeria was elevated to national park status in July 1972, and was classified as a World Heritage Site by UNESCO in 1982. The cultural richness and diversity of its natural ecosystems allowed it to become a preserve for Man and the Biosphere in 1986 (O.P.N.T, 2006). In general, the diversity of fauna found in the Tassili is the result of climatic variation, which began at the end of the last glacial period (O.P.N.T, 2006).

For their place in the Saharan and oasis ecosystems, the arthropods constitute a good biological indicator; they form a large part of essential elements for nutrition availability for numerous animal species (Beddiaf et al., 2014).

Published studies concerning the entomofauna are numerous. For example, there have been studies of the Ghardaïa region (Chouihet & Doumandji-Mitiche, 2012), the region of Adrar (Sid Amar et al., 2012), the region of Tamenrasset (Kourim et *al*, 2011; Reggani, 2010), the Djelfa region (Souttou et al., 2011), and the region of Biskra (Achoura & Bel-

hamra, 2010). However, the Djanet region has been relatively understudied given its size and ecological diversity (but see (Beddiaf et al., 2014; Chopard, 1943; Le Berre, 1989 & Dubief, 1999).

The present study consists of both qualitative and quantitative surveys of arthropods that exist in the Djanet region of Algeria over a two-season period during 2012/2013 (summer and winter). This study was designed to comprehensively assess the arthropod biodiversity of this important area by examining both natural and human disturbed habitats using three sampling methods: pitfall traps, sweep nets and yellow pan traps.

MATERIALS AND METHODS

Sampling sites

The city of Djanet is situated in the far Southeast of Algeria (Fig. 1). It is distant 420 km to the county-city Illizi and 2.200 km of the capital city Algiers. It is located in the region of Tassili N'Ajjer at 24°33' N, and 9°29' E at an altitude of 1094 m. Because of its remoteness and proximity to Libya and Nigeria, this region has not been well studied.

The region of Djanet is demarked by the Libyan border on the east, the Nigerian border along the south, the Tamenrasset Province on the southeast and by the townships of Bordj El-Houas and Illizi to the north. According to Dubief (1999), Djanet is situated on a geological anomaly that forms a cliff of sandstone that is along the occidental side of Edjeriou River. A 15 km long granitic upland separates the valley from the Tassili region. The intact sandstones of the area preserve the marks of past geological and climatic events (orogenesis, glaciation, volcanism, sea recession, sedimentation, fluvial and Aeolian soil erosion) (O.P.N.T, 2006).

Four study sites were chosen, two of them in a cultivated environment: Lokmane station, situated at 30 km South-West of Djanet with a surface area of 30 ha (Fig. 2). The second station, El Mihane, was located in the town center of Djanet with a surface area of 1 ha (Fig. 3).

Two sites located in natural environments were also selected. The first was Teghargharte station, located alongside the Teghargharte River. This station is a flood zone of Edjeriou river of Djanet. This station is found 30 km to the south of Djanet (Fig. 4).

The second natural study site (Iffoutten station) was located about 4 km from the town center of Djanet, alongside the bed-river of the Edjeriou River (Fig. 5).

We used three sampling methods for arthropod sampling: pitfall traps, sweep nets and yellow pan traps.

Analyses

Species identifications were conducted using conventional taxonomical keys, including Perrier (1927, 1940, 1980) for the Hymenoptera, Coleoptera, Hemiptera and Diptera orders and Chopard (1943) for the Orthoptera.

For this study, we used two ecological indices of composition. We calculated Total Richness and Relative Abundance for the ecological indices of composition.

Total Richness (S) corresponded to the number of species found in a given sampling area (Ramade, 1984; Blondel, 1979). The relative abundance R.A% of a species was calculated by the formula:

$$R.A \% = ni / N \times 100$$

Where ni is the percentage of a given species, and N is the total number of individuals at a given site (Dajoz, 1971; Blondel, 1979).

RESULTS

The global survey of arthropods' populations captured during two seasons of the year (summer and winter) made use of three sampling methods: pitfall traps, yellow pan traps and sweep net in the four studied stations show the presence of 191 representing 106 families, 21 orders and four classes (Table 1).

Total Richness

A total of 112 arthropods species were captured by the pitfall traps in the two environments (four stations). The two natural stations, Teghargharte and Iffoutten, were less rich with 18 and 38 species, respectively, compared to the other two cultivated stations, Lokmane and El Mihane, with 57 and 51 species, respectively.
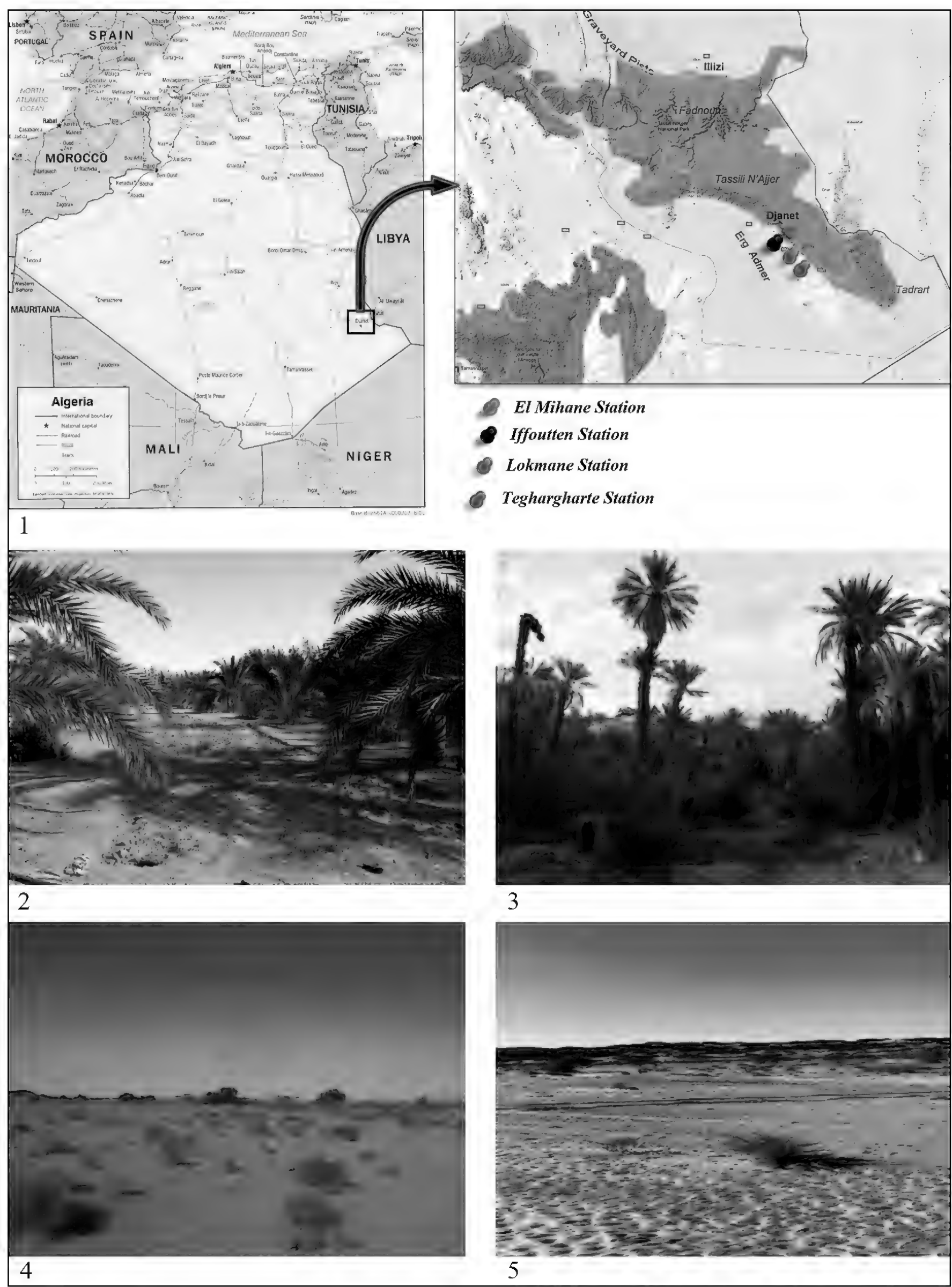
Figure 1. Geographic situation of Djanet and localization of sampling stations. Figure 2. Lokmane Station. Figure 3. El Mihane station. Figure 4. Teghargharte station. Figure 5. Iffoutten station.

Orders	Number of families	Number of species
ARACHNIDA		
Scorpiones	1	1
Araneae	6	6
Acarida	3	3
CHILOPODA		
Scolopendromorpha	1	1
COLLOMBOLA		
Entomobryomorpha	1	1
Symphypleona	1	1
INSECTA		
Odonatoptera	1	1
Blattaria	1	1
Mantodea	1	1
Isoptera	2	2
Dermaptera	1	1
Thysanoptera	1	1
Orthoptera	7	17
Hemiptera	9	14
Homoptera	5	12
Coleoptera	11	25
Hymenoptera	18	43
Nevroptera	1	1
Lepidoptera	6	9
Diptera	28	49
Aphaniptera	1	1
21	**106**	**191**

Table 1. Global survey of arthropod species captured during two seasons (summer and winter) using pitfall traps, yellow pan traps and sweep net.

The total number of arthropods species captured by the yellow pan traps was 82 species in the cultivated environment, within the two stations. We captured 39 species at the Lokmane station and 63 species at the El Mihane station.

The total number of arthropods species collected by the sweep net technique was 65 species in the cultivated environment, from both stations. These stations were similar to each other with respect to the number of species present, with 34 species for Lokmane station and 38 for El Mihane station.

Relative Abundance

Relative Abundance of Arthropod orders captured by pitfall traps during two seasons of the year is shown in figure 6.

Relative Abundance of Arthropod orders captured by pitfall traps by each station during two seasons of the year is shown in Table 2.

Relative Abundance of Arthropod orders captured by sweep net during two seasons of the year in two station is shown in figure 7.

Relative Abundance of Arthropod orders captured by sweep net during two seasons of the year in each station is shown in Table 3.

Relative Abundance of Arthropod orders captured using the yellow pan traps during the two seasons of the year is shown in figure 8.

Relative Abundance of Arthropod orders captured by the yellow pan traps during two seasons of the year in each station is shown in Table 4.

DISCUSSION

We collected a total of 112 species at the four study sites during two seasons of the year, with 57 species from Lokmane station, 51 species from El Mihane station, 18 from Taghrarte station and 38 species from Iffoutten. We note that Lokmane station is the richest in the cultivated environment and Iffoutten is the richest in the natural one. Also, we note that the cultivated habitats are more species rich than the natural environments. This may be related to denser vegetation, the availability of water, and a more moderate temperature, which may have created a less stressful environment compared to the harsher conditions in the natural environment. The natural environment is xeric, with little vegetation and is dominated by a single plant species, *Calotropis procera,* a giant milkweed.

A previous study of the Laghouat region found a total richness of 55 species in an apple orchard and 45 species along the riverbed of M'zi (Saoudi & Thelidji, 2007). These results are similar to those reported here. In contrast, our findings are higher than those found by Reggani (2010) in two cultivated environments in Tamenrasset where only eight species were collected using the same techniques.

Our results show that Hymenoptera are the most abundant order in the four studied stations. We

Stations	Cultived Stations				Natural Stations			
	Lokmane		El Mihane		Teghargharte		Iffoutten	
Orders	Ni	AR%	Ni	AR%	Ni	AR%	Ni	AR%
Scorpiones	1	0.06	0	0	0	0	1	0.17
Araneae	14	0.88	12	3.38	0	0	3	0.53
Sarcoptiformes	0	0	2	0.56	1	0.11	0	0
Scolopendromorpha	1	0.06	0	0	0	0	0	0
Entomobryomorpha	748	46.96	22	6.2	0	0	1	0.17
Symphypleona	1	0.06	0	0	0	0	0	0
Blattaria	0	0	1	0.28	0	0	0	0
Mantodea	0	0	2	0.56	0	0	0	0
Isoptera	3	0.19	3	0.85	0	0	1	0.17
Dermaptera	2	0.13	0	0	0	0	0	0
Orthoptera	32	2.01	22	6.2	0	0	1	0.17
Hemiptera	7	0.44	5	1.41	1	0.11	0	0
Homoptera	79	4.96	24	6.76	2	0.23	4	0.67
Coleoptera	12	0.75	10	2.82	25	2.85	164	27.66
Hymenoptera	675	42.37	239	67.32	849	96.7	405	68.3
Nevroptera	1	0.06	0	0	0	0	0	0
Lepidoptera	0	0	4	1.13	0	0	7	1.18
Diptera	17	1.07	9	2.54	0	0	5	0.85
Aphaniptera	0	0	0	0	0	0	1	0.17
Total	1593	100	355	100	878	100	593	100

Table 2. Relative Abundance of Arthropod orders captured by pitfall traps by each station during two seasons of the year. Ni: number of individuals. AR%: Relative abundances.

found that 67.3% and 42.4% of captured organisms were Hymenoptera at El Mihane and Lokmane stations, respectively. In the study by Chouihet & Doumandji-mitiche (2012) in the region of Ghardaïa, the arthropod communities were composed of 30.4%, 55.1%, and 41.1% Hymenoptera at the Al-Atteuf, Beni Izguen, and Dayah stations, respectively, which was similar to our findings. Additionally, Sid Amar et al. (2012) found very similar patterns to ours, where they noted a rate of 61% Hymenoptera in the palm grove of Mahdia in the region of Adrar. Our results confirm those found by Chennouf (2008) who noted values of Relative abundance for the Hymenoptera order equal to 35% in a palm grove. The order of Entomobryomorpha

is in the second most abundant group with a rate of 46.96% at the Lokmane station, and 6.2% at the El Mihane station.

However, studies by Chennouf (2008) in the Ouargla region, and Chouihet & Doumandji-mitiche (2012) in the Ghardaïa region, showed coleoptera to be the second most abundant group. Conversely, Dipterans are relatively rare with a rate of 2.54% at El Mihane station, and 1.07% at Lokman station.

CONCLUSIONS

The current Arthropodofauna study was con-

| | Cultivated stations | | | |
| Orders | Lokmane | | El Mihane | |
	Ni	A.R %	Ni	A.R %
Araneae	2	1.48	3	2.04
Sarcoptiformes	0	0	1	0.68
Mantodea	0	0	2	1.31
Odonatoptera	0	0	1	0.68
Orthoptera	10	7.24	95	62.5
Hemiptera	34	25.19	14	9.52
Homoptera	17	12.23	2	1.36
Coleoptera	55	40.74	4	2.72
Hymenoptera	4	2.96	6	4.08
Lepidoptera	3	2.22	9	5.92
Diptera	13	9.63	15	10.2
Total	138	100	152	100

Table 3. Relative Abundance of Arthropod orders captured by sweep net during two seasons of the year in each station. Ni: number of individuals. AR%: Relative abundances.

| | Cultivated stations | | | |
| Orders | Lokmane | | El Mihane | |
	Ni	A.R %	Ni	A.R %
Araneae	1	0.38	5	1
Entomobryomorpha	137	51.5	34	6.8
Blattaria	0	0	1	0.2
Thysanoptera	0	0	2	0.4
Orthoptera	18	6.77	53	10.6
Hemiptera	6	2.26	3	0.6
Homoptera	51	19.17	82	16.4
Coleoptera	0	0	3	0.6
Hymenoptera	12	4.51	63	12.6
Lepidoptera	0	0	4	0.8
Diptera	41	15.41	250	50
Total	266	100	500	100

Table 4. Relative Abundance orders captured by the yellow pan traps during two seasons of the year in each station. Ni: number of individuals. AR%: Relative abundances.
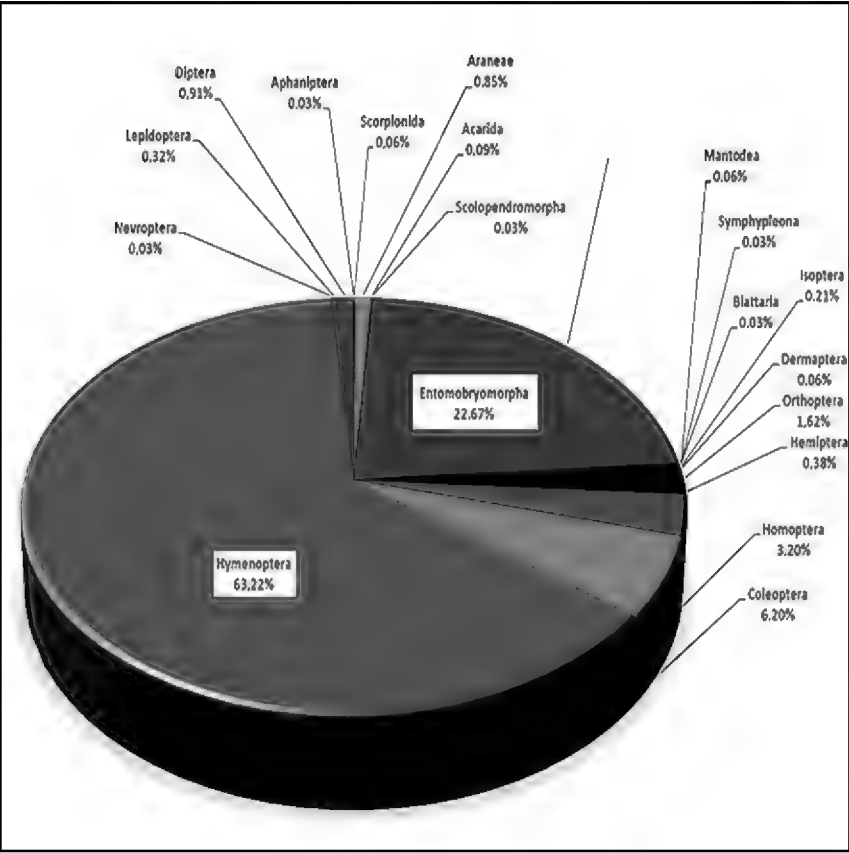
Figure 6. Relative abundances of orders of arthropods captured using pitfall traps in the four study stations.
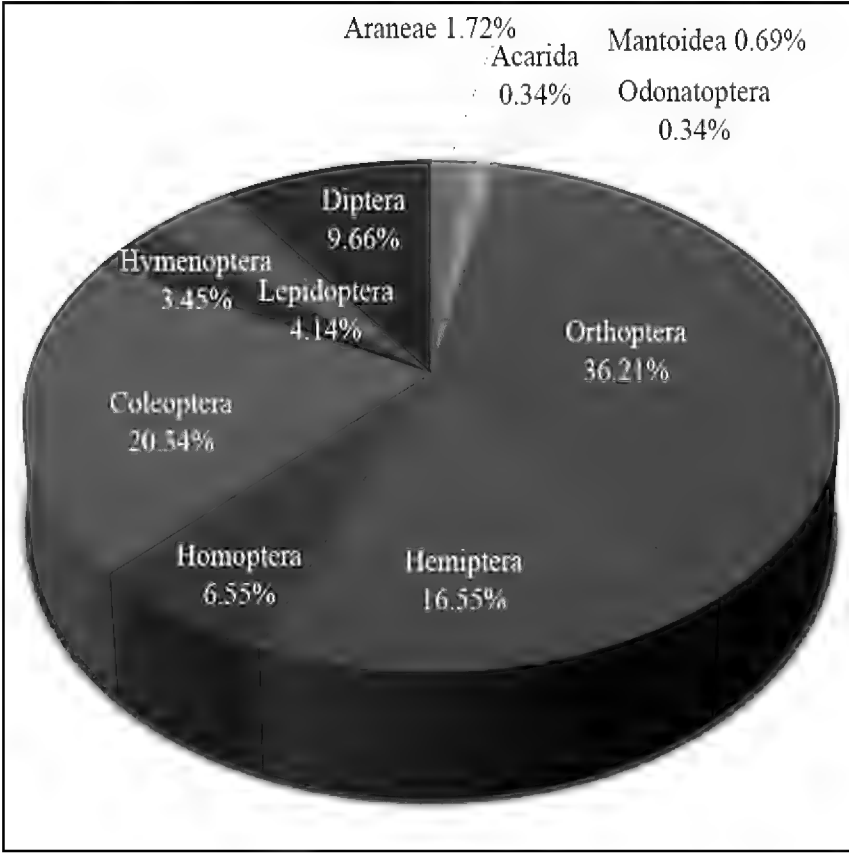
Figure 7. Relative Abundance of Arthropods species' orders captured through sweep net in two stations.

ducted in the region of Djanet in two different environments (cultivated and natural), at a total of four stations (two stations for each environment): Lokmane and El Mihane stations for the cultivated environment, and Teghargharte and Iffoutten stations for the natural environment. This study was conducted over two seasons, summer and winter. Three sampling methods were used during the two

seasons, namely: pitfall traps, sweep nets and yellow pan traps.

The pitfall trap method generated a collection of 112 arthropod species for the region. The Insecta class was the most abundant at the four stations with 10 species represented, including: *Cataglyphis bombycina* and *Anthicidae* sp. which were the most frequently captured species using this method. A
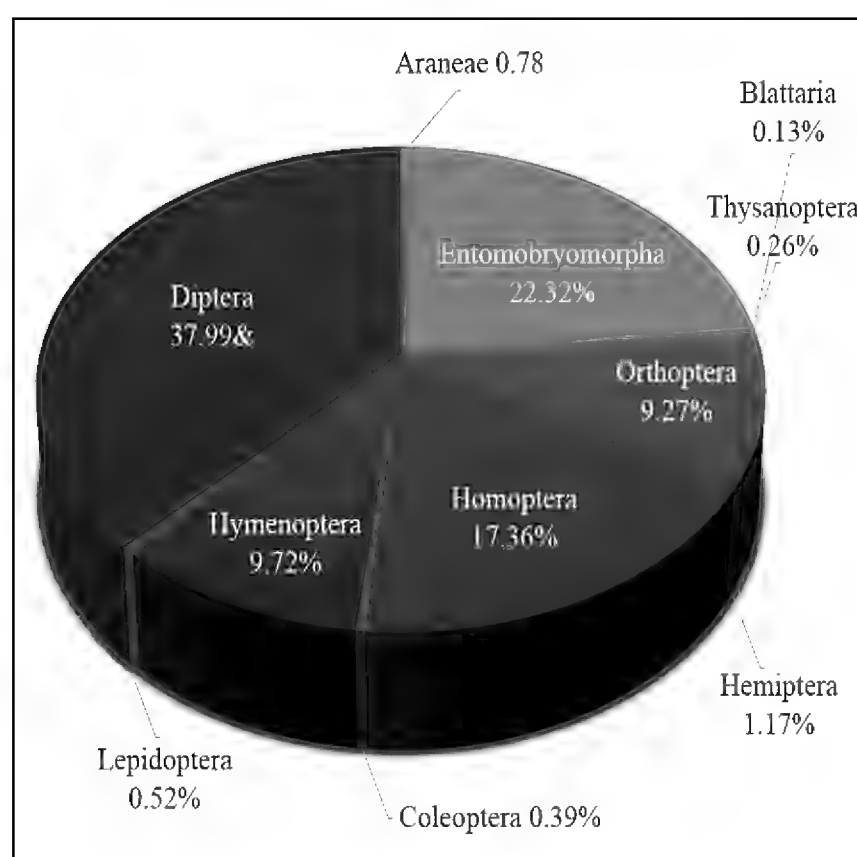
Figure 8. Relative Abundance of Arthropods species' orders captured by the yellow pan traps in two stations.

total of 2726 individuals were captured with pitfall traps during the summer and 694 individuals were captured during winter. Lokman station was ranked first with respect to the number of individuals captured by pitfall traps compared to the other stations. Hymenoptera and Entomobryomorpha orders were the most captured in the four stations using this method with relative abundance rates of 68.67% and 13.33% respectively.

The yellow pan trap method generated a total of 82 arthropod species. This method was used only in the two cultivated stations of Lokman and El Mihane. Dipterans were the most frequently captured group using this method with a rate of 40%. It is noteworthy that most of the individuals captured using this method were at El Mihane station.

The sweep net method was only used at the two cultivated stations. This sampling method allowed us to collect a large number of Orthoptera species (e.g. *Ochrilidia gracilis*), Coleoptera species (e.g., *Adonia variegatA*) and Hemiptera species (e.g. *Nysius* sp.).

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Note on occurrence of the land slug family Rathouisiidae Heude, 1885 from South Korea and its DNA barcode

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ABSTRACT   Rathouisiidae Heude, 1885 is a family of terrestrial slugs. Although only several species has so far been described in this family, rathouisiids are estimated to be highly diversified. In the present study, we report the presence of the slug of Rathouisiidae in South Korea and its DNA barcode for the first time.

KEY WORDS   Land slugs; DNA barcoding; Rathouisiidae; South Korea.

INTRODUCTION

Rathouisiidae Heude, 1885 is a family of terrestrial slugs. Although available information on this family remains insufficient at present, there are three recognized genera: (1) *Rathouisia* Heude, 1884; (2) *Atopos* Simroth, 1891; and (3) *Granulilimax* Minato, 1989 (Wu et al. 2006; Schilthuizen & Liew 2008; Kimura et al. 2020). Rathouisiid slugs are found in Australia, Papua New Guinea, Indonesia, Singapore, Malaysia, Philippines, Vietnam, Laos, Thailand, Myanmar, India, China, and Japan (Barker, 2001; Schilthuizen & Liew, 2008; Tan & Chan, 2009; Minato, 2015; Tripathy et al., 2018; Inkhavilay et al., 2019). In this family, only several species have so far been described (e.g., Wu et al., 2006; Minato, 2015). However, rathouisiids are estimated to be highly diversified (e.g., Moriguchi, 2010; Kimura in press).

While rathouisiid slugs are not recorded from the Yangtze River northward in China (i.e., from the river to the base of the Korean peninsula), they are known from the western region of Japan (Wu et al., 2006; Minato, 2015). Western Japan is geologically close to the Korean peninsula and had a land bridge to the peninsula during the last glacial period. Indeed, a lot of land gastropod genera and species are common in Japan and South Korea (e.g., Kuroda & Miyanaga, 1943; Kimura et al., 2019; Kimura & Noseworthy, 2020). However, there is so far no record of Rathouisiidae in the Korean peninsula.

MATERIAL AND METHODS

Study species and data collection

On 27 June 2018, a single specimen of the family Rathouisiidae was collected at the Bisulsan Natural Recreation Forest (35°43'41.9"N 128°32'23.8"E), Gachangmyeon, Daegu, South Korea. It was examined under a light microscope (Olympus SZ40). The examined specimen was pre-

served in the personal collections of K. Kimura (Voucher No: MNKS509).

Total DNA was isolated from a foot piece of the individual using Nucleospin tissue (TaKaRa, Shiga Pref., Japan) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced. The condition used for the polymerase chain reaction (PCR) followed the protocol described by Kimura et al. (2020) and the primer set LCO1490 (5'-GGTCAACAATCA-TAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATC-3') was used (Folmer et al., 1994). The PCR product was purified using Exo-SAP-IT (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Sequencing was performed using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and electrophoresed using an ABI 3130xl sequencer (Applied Biosystems, Carlsbad, CA, USA). The resulting COI sequence has been deposited in the DDBJ/EMBL/GenBank database (Accession No: LC598438). In addition to this new sequence of the Korean rathouisiid slug, already existing data of COI gene of Rathouisiidae and its sister family Veronicellidae were obtained from GenBank to conduct a phylogenetic analysis (Table 1).

Phylogenetic analyses

These sequences were aligned with MUSCLE v3.8 (Edgar 2004). The phylogenetic tree was obtained for a COI gene data set (581 sites) using the maximum likelihood (ML) method. Evolution model selection and generation of a ML tree were conducted with MEGA (Kumar et al., 2018). As a result of the model selection, the GTR+G+I model was selected. For the ML tree obtained, we assessed nodal support by performing bootstrap analyses with 1000 replications. The species of Veronicellidae was used as the outgroup.

RESULTS AND DISCUSSION

Systematics

Phylum MOLLUSCA Cuvier, 1797
Classis GASTROPODA Cuvier, 1795
Ordo SYSTELLOMMATOPHORA Pilsbry, 1948
Familia RATHOUISIIDAE Heude, 1885

Rathouisiidae sp.

DESCRIPTIONS. Long and slender. Measurements: length 14.6 mm, width 2.1 mm (Fig. 1, Voucher No: MNKS509). Notum round, lacking a dorsal keel, pale brown, covered with circular granules. Upper tentacles short, black. Lower tentacles pale grey, with a two-lobed shape. Foot with a narrow sole. A small groove between the footsole and each hyponatum. Male genital opening situated at the base of the right lower tentacle. Female genital

Figure 1. The rathouisiid slug used in this study. Voucher No MNKS509. Scalebar: 1.0 mm.

FAMILY	SPECIES NAME	LOCALITY	VOUCHER #	GENBANK
Rathouisiidae	Rathouisiidae sp.	Gachangmyeon, Daegu, South Korea	MNKS509	*LC598438
Rathouisiidae	*Granulilimax fuscicornis*	Tokushima, Japan	KC4519	LC508386
Rathouisiidae	*Granulilimax* sp.	Kuchinoshima Isl., Kagoshima, Japan	HC2897	LC522963
Rathouisiidae	Rathouisiidae sp.	Okinawa, Japan	KC9179	LC508385
Rathouisiidae	Rathouisiidae sp.	Izena Isl., Okinawa, Japan	HC4738	LC522965
Rathouisiidae	Rathouisiidae sp.	Amamioshima Isl., Kagoshima, Japan	HC2305	LC522962
Rathouisiidae	Rathouisiidae sp.	Kikai Isl., Kagoshima, Japan	HC3981	LC522964
Rathouisiidae	*Atopos* sp.	Batang Padang, Perak, Malaysia	Rathoui03	LC522961
Rathouisiidae	*Atopos* sp.	Myanmar	FLMNH494081	MF983573
Rathouisiidae	*Atopos* sp.	Myanmar	FLMNH494022	MF983574
Rathouisiidae	*Atopos* sp.	Myanmar	FLMNH494195	MF983575
Veronicellidae	*Laevicaulis natalensis*	South Africa	NM-W1444	HQ660051
Veronicellidae	*Laevicaulis* sp.	South Africa	NM-W4061	HQ660052
Veronicellidae	*Laevicaulis alte*	-	-	MN022749
Veronicellidae	*Phyllocaulis tuberculosus*	Brazil	MCP 8857	HQ660053
Veronicellidae	*Phyllocaulis variegatus*	Brazil	CASIZ 180487	HQ660054
Veronicellidae	*Vaginulus taunaisii*	Brazil	MCP 8858	HQ660056
Veronicellidae	*Veronicella cubensis*	Hawaii	CASIZ 180489	HQ660057

Table 1. List of the species included in the molecular phylogenetic analysis.
Asterisk indicates the sequence newly obtained in this study.

opening on the foot groove, situated somewhat behind the head. Anal pore on the foot groove, slightly anterior to the female genital opening. Pulmonary orifice on the foot groove, slightly posterior to the female genital opening.

DISTRIBUTION AND BIOLOGY. This Korean rathouisiid was so far only encountered in deciduous broad-leaved forests at the Bisulsan Natural Recreation Forest, Gachangmyeon, Daegu, South Korea. The slug individual was observed in the leaf litter layer.

MOLECULAR PHYLOGENY. The result of the phylogenetic analysis (Fig. 2) suggested that the Korean rathouisiid slug reported in this study belongs to neither *Atopos* in Southeast Asia (clade C) nor *Granulilimax* in Japan (clade B). Moreover, it was supposed that the slug is, to some extent, genetically distinct from the clade containing the remaining Japanese species (clade A). The Korean rathouisiid may belong to the remaining already-recognized genus *Rathouisia*, whose COI barcode was not available. Additional specimens of Rathouisiidae are needed to examine this possibility.

REMARKS. The specimen examined here had an undeveloped male reproductive organ and was an immature individual. Therefore adult body size and shape of developed reproductive organs remain to be elucidated.
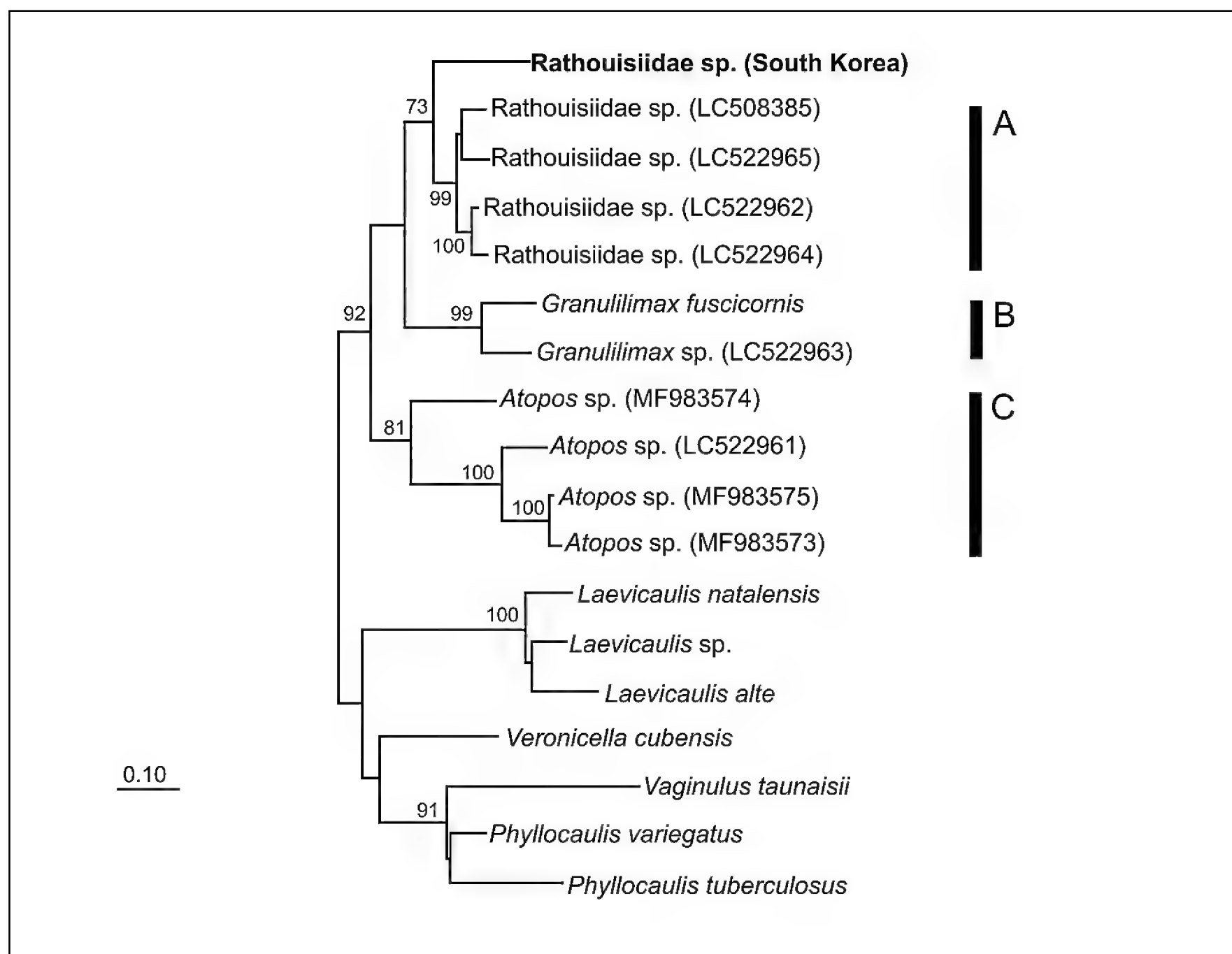
Figure 2. Maximum likelihood tree of the rathouisiid slugs based on 581 bp of the COI gene. Each OTU label represents a species name and the Korean specimen is in bold. Numbers on branches indicate maximum likelihood bootstrap values. Scale bar indicates 0.10 substitutions per site.

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Diet study of Atlantic horse mackerel (*Trachurus trachurus* Linnaeus, 1758) (Carangiformes Carangidae) caught in Béni-Saf Bay, Western Mediterranean Sea (Algeria)

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ABSTRACT	This paper gives a description of the diet of Atlantic horse, *Trachurus trachurus* (Linnaeus, 1758) (Carangiformes Carangidae) living in the bay of Béni-Saf (North-Western Algeria) between November 2015 and October 2016 by analyzing the contents of the stomachs of 488 specimens divided in three classes according to the length of their body. We used several indices such as the coefficient vacancy (V), the percentage of prey (Cn) and the frequency of occurrence of prey (Fc). Of the total stomachs examined, 135 are empty which corresponds to a vacancy coefficient of 27.66. In total, 3912 preys were counted. They belong to ten major taxonomic groups: Copepoda, Euphausiacea, Amphipoda, Mysidacea, Cumaceae Cephalopods, Echinodermata, Molluscs, phytoplankton and Teleosts). The study of diet of *T. trachurus* shows that it has a broad food spectrum, and is a carnivorous and voracious species (benthpelagic). We found that the first class prefers Crustaceans, the second favors Euphausiacea, and the third prefers the predation of Teleosts. The diet varies with the size of specimens which is proportional to the size of the prey.

INTRODUCTION

Understanding the biology and ecology of fish in the wild inevitably involves studying their diet. The analysis of the contents of the stomach or of the entire digestive tract makes it possible to determine indirectly the composition of the diet and to assess the use of the food available. Processing of the collected data can be done by calorimetric (Rosecchi & Nouaze, 1985), numerical, gravimetric, volumetric or occurrence methods (Hyslop, 1980). These different methods have given rise to food indices, which show the most important prey for the predator's diet and allow inter-specific and intra-specific comparisons. Some authors group together the prey of a studies species, according to their index value, in order to separated the main prey from the others (Zander, 1982). It is in this context that our research falls, namely the study of the diet of the horse mackerel, *Trachurus trachurus* (Linnaeus, 1758) (Carangiformes Carangidae) caught in the west coast of Béni-Saf in western Algerian. We used food indices which tell us about the proportions of prey ingested and group them into "categories".

Horse mackerel has been the subject of several studies on reproduction (Korichi, 1988; Tahari,

2011; Aydin & Erdoğan, 2018; Gherram, 2019; Rahmani & Koudach, 2020), growth (Karlou-Riga & Sinis, 1997; Abaunza et al., 2003; Kerkich et al., 2013), and diet (Olaso-Toca et al., 1999; Cabral & Murta, 2002; Jardas et al., 2004; Šantić et al., 2005; Bahar & Tuncay, 2009; Bayhan et al., 2013; Shawket et al., 2015). However, for the Algerian coasts, no important study has been carried out.

This paper focuses on the diet of Atlantic horse, *T. trachurus*, living in the Béni-Saf Bay (North-West Algeria) with emphasis on vacuity coefficient, the Corrected frequency index of a prey (FC) and the Percentage in number of a prey (Cn%) to complete gaps in life cycle of this Carangidae and better manage this resource in that part of the Algerien coast.

MATERIAL AND METHODS

The samples of hores mackerel (*Trachurus trachurus*) come from commercial catches landed in the port of Béni-Saf in the western Mediterranean Sea, between the Habibas islands to the east and the tip of Rachgoun to the west, between longitude 1° 10 and 1° 30 West (Fig. 1). Sampling was carried out monthly between November 2015 and October 2016. The individuals come from trawls (bottom and beam trawls) carried out at a depth of 30 and 130 m. For each individual we noted the height and the weight.

They have been divided into three size classes:

• Class 1: total length ≤ 12 mm Small.
• Class 2: 12 cm <total length <20 cm Medium.

Figure 1. Geographical localization of the study area: Béni-Saf Bay, Algeria.

• Class 3: total length ≥ 20 cm Large.

The individuals are then dissected in order to recover the entire digestive tract immediately after capture, and then the stomach contents are stored in a formalin solution (10%). The ingested preys are identified with a binocular magnifier up to the family level, then under the microscope to possibly continue until the species. The excessively altered remains were classified in the indeterminate group. We used several identification keys (Rioja & Lo Bianco, 1928; Pasteur-Humbert, 1962; Bellon-Humbert, 1973; Kensley, 1978; Ruffo, 1982, 1989; Ledoyer, 1983; Sarda, 1984; Fischer et al., 1987a, b).

The stomach contents are grouped by month and by season, to obtain representative samples. These groups are: October, November and December in autumn, February-March in winter, April, May and June in spring and July, August and September in summer. To deepen the study of different diets and their variations, we used the mixed method (qualitative and quantitative), as well as different dietary indices.

Sex ratio

It is defined as being the proportion of the male or female individuals compared to the total number of individuals. It also gives an idea on the balance of the sexes within the population. The sex-ratio generally translates the rate of femininity or masculinity of the population:

SR = F/(M+F)*100 (Kartas & Quignard, 1984)

F = number of females; M = number of males.

Vacuity coefficient (V)

It is the ratio (in percentage) of the number of empty stomachs to the total number of stomachs examined. This coefficient makes it possible to identify, over time, the periods of weak and intense trophic activities in fish.

V = NEV / NET

NEV: number of empty stomachs.
NET: total number of stomachs.

The Corrected Frequency Index of a prey (FC)

According to Gray et al. (1997), it is calculated as follows:

$$FC = Fi / \sum Fi * 100 \text{ with } Fi = ni \, P_i$$

$Fi =$ the frequency index of a prey.
$ni =$ number of stomachs containing the prey i.
$NP =$ number of number of stomachs containing the prey i.

Percentage in number of prey (Cn)

It is the ratio (in percentage) of the total number of individuals of a prey p on the total number of the various prey ingested per batch of n fish. (Table 1).

$$Cn = 100 * Pi / P$$

$Pi =$ total number of individuals of a prey.
$P =$ the total number of various prey.

RESULTS

In total, 488 specimens of *T. trachurus* were collected (Fig. 2), 240 males (49.18%), 208 females (42.62%) and 40 unsexed (8.20%).

The length frequency distribution of the entire population is shown in figure 2, male length range was 10.2 to 34.4 cm, female length range was 9.1 to 35.1 cm while males weight varied between 11.38 and 301.27 g and females' weight varied between 5.09 and 343.41 g.

Sex Ratio

From a 12-month sample represented by a workforce of 488 individuals, we obtained a total masculinity rate of 53.57% for a total femininity rate of 46.43% (Fig. 3). Overall, in our sample, there is a convergence between the two sexes ratio with a slight favor of males. In addition, the variations in sex ratio according to size is in favor of females whose total length is less than 16.5.

In sizes between 16.5 and 35.5 cm, males outnumbered females, except for size 34.5 cm and 35.5 cm, where females outnumbered males. Monthly variations of sex-ratio (Fig. 3) reveal that females
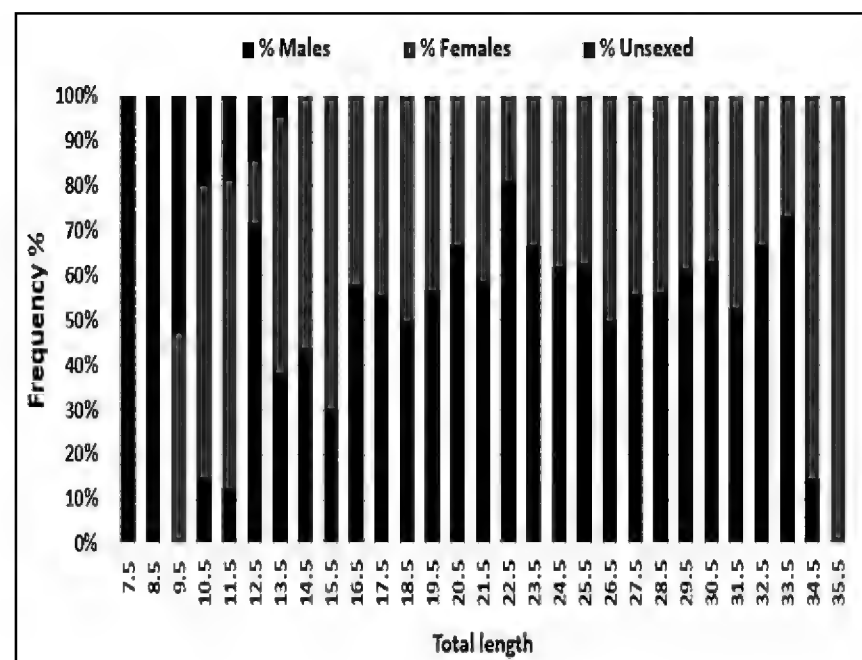
Figure 2. *Trachurus trachurus* length frequency distribution of males and females caught in Béni-Saf Bay.
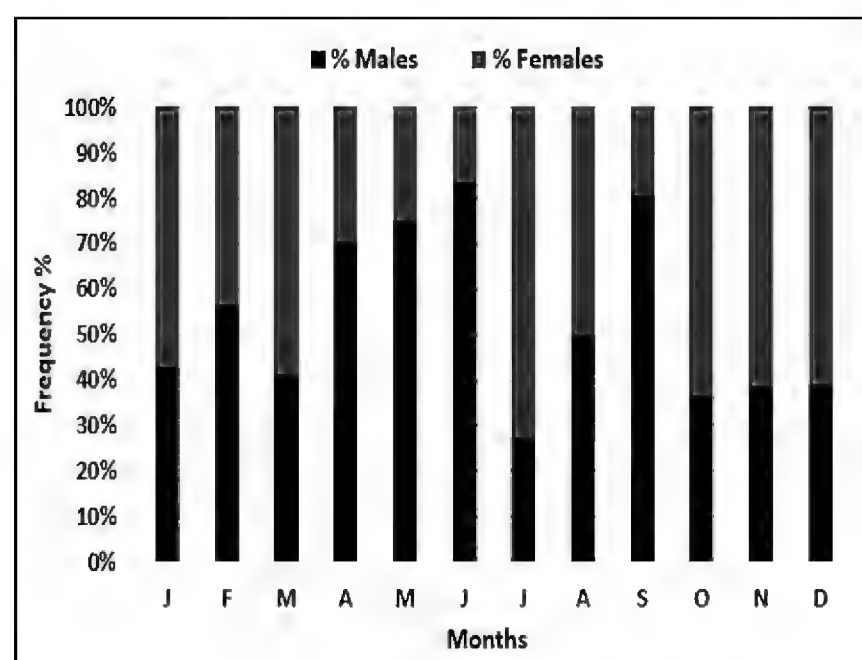
Figure 3. Monthly evolution of Sex ratio of *Trachurus trachurus* caught in Béni-Saf Bay.
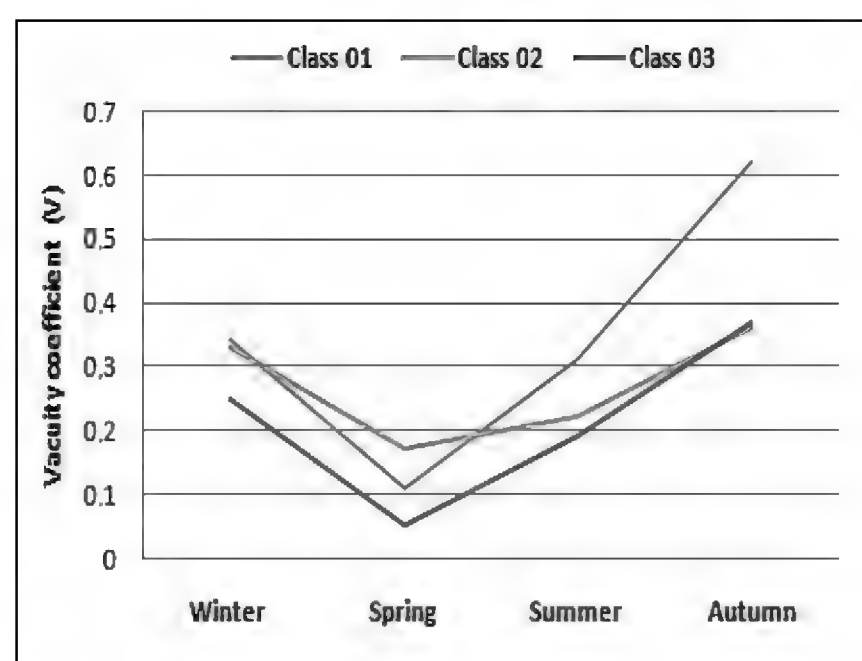
Figure 4. Seasonal variation of vacuity coefficient of *Trachurus trachurus*.

dominate during the months of January, March, July, October, November and December. And males dominate during February, April, May, June, and September, with a numerical equality in August. Evolution of sex-ratio related to seasons (Fig. 5) showed that females dominate in the winter period while males dominate in the spring-summer-autumn period.

Vacuity coefficient (V)

The Vacuity coefficient shows seasonal fluctuations (Fig. 4). This coefficient displays the lowest values during the spring season, which is explained by the abundance of prey in the environment. While it marks the highest values in the autumn period, which indicates the scarcity of prey in the environ-

Cn%	Fc%	Category of Prey
Cn > 50 Main prey	FC > 30: Preferential	Main Preferential
	FC < 30: Occasional	Main Occasional
10 < Cn < 50 Secondary prey	FC > 10 : Frequent	Secondary Frequent
	FC < 10: Accessory	Secondary Accessoire
1 < Cn < 10 Complementary prey	FC > 10: 1st Order	Complementary 1st Order
	FC < 10: 2nd Order	Complementary 2nd Order
Cn < 1 : accidental prey	accidental prey	accidental

Table 1. Classification of prey according to Cn and Fc (Hureau. 1970).
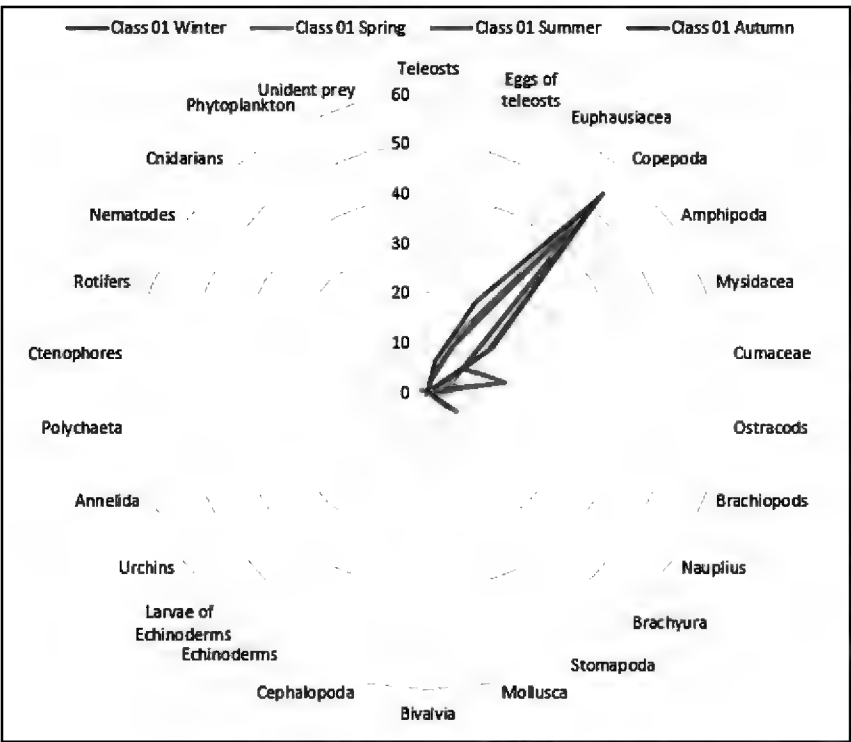
Figure 5. Seasonal composition of the diet of *Trachurus trachurus* (class 1) caught in Béni-Saf Bay.

ment, which leads to feeding difficulties and cause the fish to eat less.

Composition of the diet according to the season

Class 1

The food spectrum included 8 preys in winter, 14 in spring, 7 in summer and 5 in autumn (Fig. 5).

In winter, Copepoda are the main prey. Amphipoda, Cumaceae and Euphausiacea are secondary prey. The Nauplius and eggs of Teleosts constitutes the complementary prey of 2nd order. The rest are accidental prey. In spring, prey appears more diverse. The Copepods constitute the main prey, the Euphausiacea constitute the secondary prey. Amphipods, Cumaceae and *Nauplius* are complementary prey (1st order). The Mysidacea, squid and eggs of Teleosts are complementary prey (2nd order). The remains of Brachiopods, Echinoderms and Mollusc are accidentally preyed. In summer, the consumption is focused on Crustacea, but there is no main prey. The Euphausiacea and the Amphipods constitute the secondary accessory prey, the eggs of Teleosts and Nauplius are complementary prey (2nd order). There is no accidental prey. In Autumn, the foods of this fish is based on the Copepods which constitute the main prey. Euphausiacea and Amphipods take a more important place but remain secondary prey. Eggs of Teleosts are complementary prey. There is no lack of acidental prey.

Class 2

The food spectrum includes 12 preys in winter, 20 in spring, 11 in summer and 8 in autumn (Fig. 6).

In winter there is no main principal prey. Compared to the first class, Euphausiacea occupy a large place. The decrease in the consummation of Copepods was offset by an increase in that of Euphausiacea which then became the preferred prey of this size class, but it remains classified in the category of secondary prey. In addition, we note the emergence of a new prey, Small fish (Teleosts) and squid as secondary prey. Amphipods are complimentary prey (2nd order). We have also recorded the appearance of Stomapods, Bivalvia, and larvae of Echinoderm as accidental prey.

In spring, the fish keep the same winter feeding behaviour with the appearance of new preys. The Ostracoda, the Rotifers and Bivalvia as complementary prey (2nd order). The rest (crabs, Molluscs, echinoderm, Polychaeta, Ktenophores, Cnidarians, phytoplancton) constitute accidental prey.

During summer and autumn the fish maintain the same eating behaviour with slight differences, such as a higher predation of squid in autumn. Molluscs and Cnidarians appear as both accidental and new prey in summer.

Class 3

The food spectrum included 13 preys in winter, 20 in spring, 10 in summer and 8 in autumn (Fig. 7).

In winter, consumption is focused on the Small fish (Teleosts) which constitute the main prey, while Euphausiacea are secondary accessory prey. Our results show a significant reduction in Crustaceans considered complementary prey after having been a staple food of the two previous classes. The diet has also seen the emergence of a new prey of bivalvia as a complementary prey of the 2nd order, the rest of the food represents accidental prey.

In spring and summer, the diet is almost identical; it does not contain main prey. In both seasons, it prefers to feed on Small fish but remain classified as secondary prey, followed by predation of Mysidacea and squid. The difference between the two seasons shows that the spring diet is more varied and results in the emergence of a new prey; the Stomapods as complementary prey (2nd order).

In autumn,, the diet is entirely dominated by Small fish (Teleosts) (preferential primary prey), Euphausiacea and Amphipods are frequent secondary prey. The rest are accidental preys.

DISCUSSION

The diet study of *T. trachurus* has shown that this species has a wide food spectrum, and is an omnivorous and euryphagous fish. Its food is dominated by benthic (mainly Crustaceans) and pelagic (Copepods, Teleosts) prey, which is confirmed by (Jardas et al., 2004; Šantić et al., 2005; Bahar & Tuncay, 2009; Cabral & Murta, 2002; Bahar et al., 2013; Shawket et al., 2015; Koç & Erdoğan, 2019).

The annual vacancy factor is 22.55% (110 empty stomachs), and we notice that it decreases with an increase in the size of individuals, as the highest values were recorded in young individuals and this is consistent with Shawket et al. (2015) and Koç & Erdoğan (2019). The low vacancy factor during the spring and summer is due to the availability of prey. Also, the nutritional activity may be affected by physiological and ecological phenomena (such as reproduction and migration).
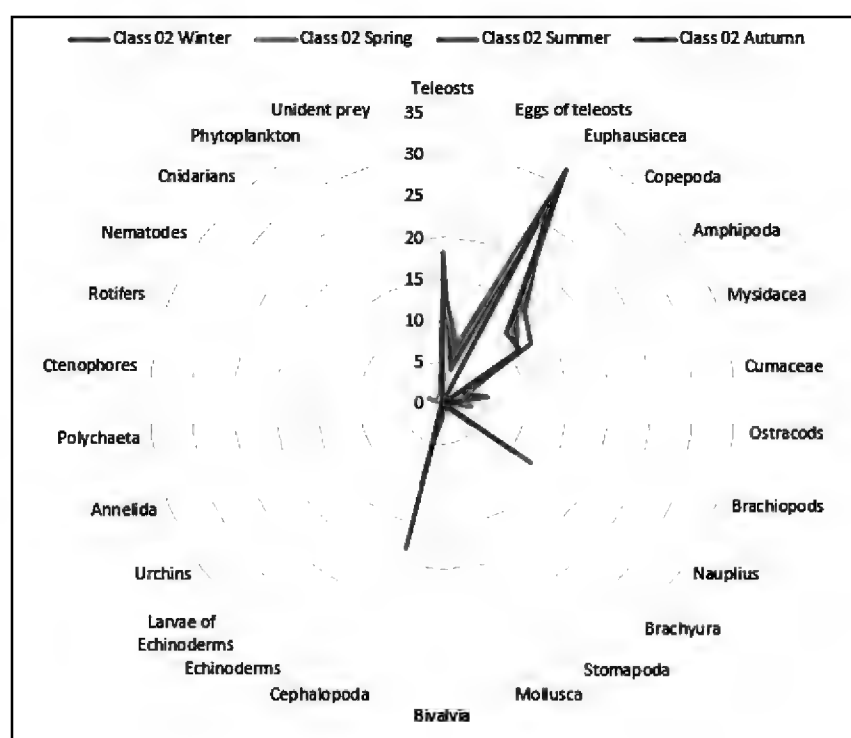
Figure 6. Seasonal composition of the diet of *Trachurus trachurus* (class 2) caught in Béni-Saf Bay.
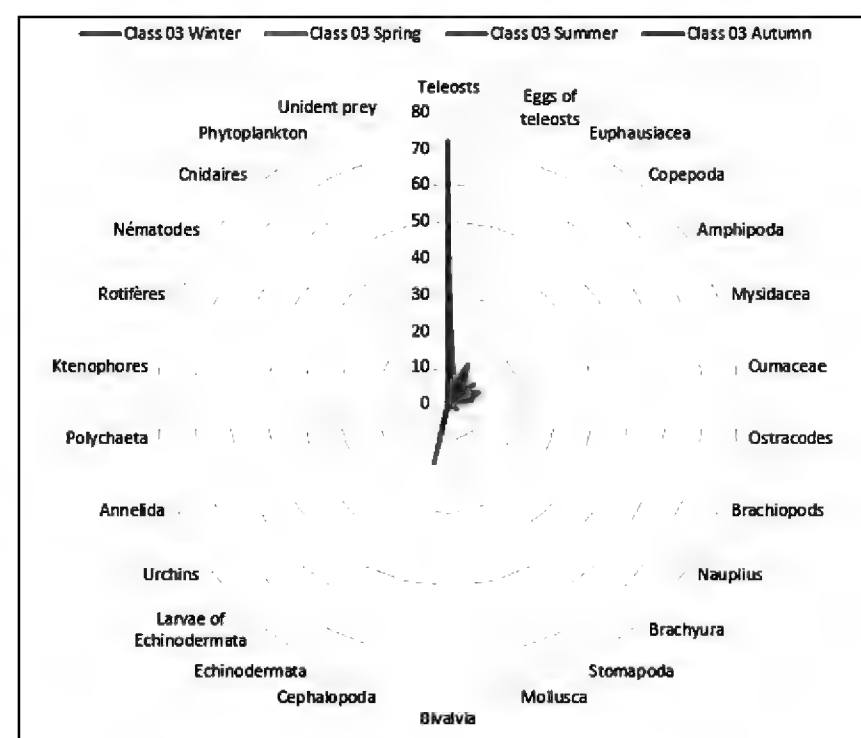
Figure 7. Seasonal composition of the diet of *Trachurus trachurus* (class 3) caught in Béni-Saf Bay.

As the size class increases, the qualitative and quantitative composition of the food evolves, and the dietary diversity in the large *T. trachurus* suggests that they exploit a wide range of prey. The selection of the types of prey is in relation to the size of the predators (Chapman et al., 1988; Bayhan & Tuncay, 2009; Shawket et al., 2015;), the size classes of *T. trachurus* exploit different types of prey, which would decrease the competition between small and large individuals, which is confirmed by Bănaru & Harmelin-Vivien (2009).

The composition of the diet of this species is based on six major taxonomic groups (Euphausiacea, Copepoda, Amphipoda, Mysidacea, Cumaceae and Teleosts), which is confirmed by several authors (Kompowski, 1976; Šantić et al., 2005; Bănaru, 2008; Bahar & Tuncay, 2009; Bahar et al., 2013; Koç & Erdoğan, 2019). The presence of supra-benthic taxa (Stomatopoda, Bivalvia, Echinoderma, cephalopoda, Brachyura) indicates, however, their expediency or aptitude to use sometimes more resources at the bottom. This fish shows a great plasticity of its diet and can feed whatever the environment, which is consistent with the study of Bănaru (2008).

The results that we obtained in this study show

Cn %	Class 01				Class 02				Class 03			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Teleosts	0	0	0	0	18.29	13.94	15.56	14.84	53.06	41.61	40.46	71.7
Eggs of teleosts	3.16	2.21	2.48	6.42	4.18	7.31	5.66	0.23	0.47	5.8	2.93	3.02
Euphausiacea	12.69	13.11	10.34	20.67	27.26	31	29.05	31.86	11.77	11.69	10.32	9.94
Copepoda	51.11	53.4	49.01	52.04	14.71	13.5	12.56	11.36	4.77	6.59	5.39	2.14
Amphipoda	14.67	8.53	8.34	15.96	12.8	10.75	10.88	11.28	8.7	4.27	5.6	5.02
Mysidacea	1.8	5.25	10.97	0	2.89	0.63	3.5	0	0.3	8.31	8.99	0
Cumaceae	10.64	6.1	15.68	0	5.47	2.5	2.26	2.08	6.51	0.82	5.88	0
Ostracodes	0	1.73	0	0	0	3.44	0	0	0	0,3	0	0
Brachiopods	0	0.12	0	0	0	0	0	0	0	0	0	0
Nauplius	5.54	6.89	2.94	4.91	3.15	4.38	12.77	10.36	1.22	2.23	2.77	0.26
Brachyura	0	0	0	0	0.1	0.81	0	0	0.43	0.42	0	0.61
Stomapoda	0	0	0	0	0.88	0	0	0	0	1.56	0	0
Mollusca	0.11	0.03	0	0	0	0.22	0.1	0	0.06	0.72	0	0
Bivalvia	0	0.09	0	0	0.1	1.66	0	0	1.1	0.96	0	0
Cephalopoda	0	0	0	0	10,12	5.66	7.51	17.99	11.13	13.2	16.98	7.31
Echinodermata	0	0	0	0	0	0,06	0	0	0	0	0	0
Larvae of Echinodermata	0	0.54	0	0	0.05	0.28	0	0	0	0.18	0	0
Urchins	0	0.06	0	0	0	0	0	0	0	0	0	0
Annelida	0	0	0	0	0	0	0	0	0	0.18	0	0
Polychaeta	0	0	0	0	0	0.28	0	0	0	0	0	0
Ktenophores	0	0	0	0	0	0.13	0	0	0	0	0	0
Rotifères	0	1.49	0	0	0	1.66	0	0	0	0.3	0	0
Nématodes	0	0	0	0	0	0	0	0	0.3	0.66	0	0
Cnidaires	0	0	0	0	0	0,03	0.15	0	0	0.06	0.05	0
Phytoplankton	0	0	0	0	0	0.88	0	0	0	0.14	0	0
Unident prey	0.28	0.45	0.24	0	0	0,88	0	0	0,18	0	0.63	0

Table 2. Composition of the diet of *Trachurus trachurus* according to Cn%.

that the main prey of the horse mackerel of the Bay of Béni-Saf are crustaceans for a size of fish lower than 20 cm. Beyond this size (TL 20 cm) Small fish (Teleosts) are the preferred prey.

CONCLUSIONS

Our study has shown that the horse mackerel *T. trachurus* of Béni-Saf bay (North-Western Mediterranean Sea) is a carnivorous species (benthopelagic), whose food spectrum consists mainly of Crustaceans (Copepods, Euphausiacea, Am-phipods, Mysidacea, Cumaceae), Cephalopods and vertebrae (Teleosts and Eggs of teleosts). In the first two size classes (Lt <20 cm), Crustaceans constitute the most important group of prey, for class 3 (Lt> 20 cm), Teleosts (anchovies, sardines) constitute the group most preferable prey. We conclude that the diet of this fish is very flexible as it changes prey in proportion to predation capacity and environmental conditions.

There is little seasonal variation in the diet, crustaceans were the dominant prey in all seasons for the first two size classes, while fishes were the dominant prey in class 3 (Table 3).

	Class 1	Class 2	Class 3
Teleosts	---	Secondary Frequent	Main Preferential
Eggs of teleosts	Complementary 1st Order	Complementary 2nd Order	Complementary 2nd Order
Euphausiacea	Secondary Frequent	Secondary Frequent	Secondary Frequent
Copepoda	Main Preferential	Secondary Frequent	Complementary 2nd Order
Amphipods	Secondary Frequent	Secondary Frequent	Complementary 1st Order
Mysidacea	Complementary 2nd Order	Complementary 2nd Order	Complementary 2nd Order
Cumaceae	Complementary 1st Order	Complementary 2nd Order	Complementary 2nd Order
Ostracods	accidental	accidental	accidental
Brachiopods	accidental	---	---
Nauplius	Complementary 2nd Order	Complementary 2nd Order	Complementary 2nd Order
Brachyura	---	accidental	accidental
Stomapoda	---	accidental	accidental
Mollusca	accidental	accidental	accidental
Bivalvia	accidental	accidental	accidental
Cephalopoda	---	Secondary Frequent	Secondary Frequent
Echinoderms	---	accidental	---
larvae of Echinoderms	accidental	accidental	accidental
Urchins	accidental	---	---
Annelida	---	---	accidental
Polychaeta	---	accidental	---
Ctenophores	---	accidental	---
Rotiferes	accidental	accidental	accidental
Nematods	---	---	accidental
Cnidarians	---	accidental	accidental
Phytoplankton	---	accidental	accidental
Unident prey	accidental	accidental	accidental

Table 3. Categories of preys ingested by horse mackerel (*Trachurus trachurus*).

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Impact of Nematode *Heterodera avenae* Wollenwebwer, 1924 (Heteroderidae) attack on cereal yields in the region of Tiaret (Algeria)

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ABSTRACT   The cyst nematode *Heterodera avenae* Wollenweber, 1924 (Heteroderidae) is an obligate endoparasite of grasses. It is the most studied and most damaging nematode known for more than a century. The study of the distribution of the *H. avenae* cyst nematode was carried out on some plots of cereal-oriented municipalities in the Tiaret region to assess the infestation levels of the plots in order to study the impact of the nematode infestation on cereal yields. Spearman's test was used, which allowed us to study the correlation between the degree of infestation and cereal yields, particularly wheat, barley and oats. The plots of the communes surveyed are all infested by H. avenae with different levels of infestation except the plots of the town of Sebaine and Mahdia where the infestation is practically non-existent (0 cysts/100 g of soil). The correlation is negative between the degree of infestation and the yield (r = -0.06), the yields of hard wheat are low when the degree of infestation is high. Soft wheat and barley yields are negatively correlated with respectively, r = - 0.26 and r = -0.27, therefore, heavy infestations of the nematode lead to a decrease in yield. Unlike other cereal species, the correlation is positive with the degree of infestation (r = 0.18), so the infestation of the nematode did not greatly influence oat yields. Oats are a tolerant species for nematode attack in relation to wheat and barley that have recorded decreases in yields. Unlike the three previous crop types, oats stand out for their tolerance to this parasite.

INTRODUCTION

The main crops in Algeria are cereals. Their importance is justified through a wide use of their products that are the most consumed. With a population in perpetual growth, the low yields may not satisfy the National needs despite subsidies from the state.

At the level of the high plateaus, grain farming is the most practiced particulary in the region of Tiaret formerly called the attic of Algeria. Among the obstacles that impede the farming of cereals, we can mention the climatic vagaries (the variation in temperature and low rainfall).

Added to this, the bio-aggressors which weaken the productions of cereals, including the nematodes pests. The losses of agricultural productions due to nematodes have been estimated at 10% of the world production (Whitehead, 1998).

This species was found in many countries like Australia, South Africa, Japan, and most European countries (Kort, 1972), India (Sharma & Swarrup, 1984), West Asia and Pakistan (Sikora, 1988) Saudi Arabia (Ibrahim et al., 1999). In North Africa, *Heterodera* was found in Algeria by Scotto La Massesse (1961), Lamberti et al. (1975) and Mokabli et al. (2001), in Morocco by Franklin (1951) and Rammah (1994), and in Tunisia by Ritter (1959); most of the works devoted to it are limited to Europe, Australia, and India (Swarup & Sosa-Moss, 1990).

In Algeria, the losses caused by this nematode are not well known. Indeed, the lack of data on this species and its losses justify the choice of the topic of the study, which focuses on the cyst nematode *H. avenae* in the region of Tiaret.

The present work is devoted to the study of the distribution of the nematode to cysts *H. avenae* on some plots of certain common to grain vocation of the region of Tiaret to assess the degrees of infestation of parcels in order to study the impact of the infestation of the nematode on the yields of cereals.

MATERIAL AND METHODS

Nematological analysis is based on a sample which consists of the search and counting of nematode in the cyststate. Extraction of cysts has been done with the help of Fenwick methods (Fenwick, 1940) modified by Oostenbrink (1950) (see also Sosa-Moss, 1966). Regions cultivated in cereals have been chosen according to the infestation level and after theprevious investigation. Samples consist to remove quantities of ground with depth from 10 to 40 cm. This has been done at a rectangular level area of 12 m² (2.40 x 5 m).

The extraction apparatus of cysts is constituted by a body measuring 30 cm height, 15 cm diameter at the bottom and around of 9 cm at the top. Apparatus's bottom is constituted by an oblique plan sloping to an orifice allowing emptying, is surmounted by a funnel supporting a kitchen colander with stitches of 1 mm, and the upper part of the apparatus body is rounded with a sloping gutter that allows the water contained in the cysts to flow out.

A sifter of 250 µm to gather afterwards cysts and certain organic fragments. According to Sosa-Moss (1966), the functioning principle of Fenwick apparatus is founded on floating and density of cysts more or less dry according to the water.

Density scale is used as follows: Dry cyst <water (1) <nematode (1.08–1.09) <wet cyst (1.18 –1.22) <high density solution <mineral material.

Container is full of water; we put down withered ground in the stitched colander of 1 mm which retains the biggest items while thin particles and cysts are pushed away by the water jet; clays, thin organic fragments and cysts, floating by impulse of water current, are carried away into the gutter by overflowing of the suspension which flows out on a sifter of 250 µm between stitches; this sifter collects only cysts and some fragments. The part of suspension retained on the sifter of 250 µm is gathered in blotting paper under a funnel which releases excess water. With help of a double magnifying glass, cysts are harvested by a brush or by a fine needle.

RESULTS

The study conducted at the level of a few towns in the region of Tiaret reveals the degrees of various infestation ranging from the total absence of the nematode to infestations that largely exceed the threshold of harmfulness (Table 1).

The attack of the nematode affects the cytology and the physiology of the plant. This has a direct impact on the development of the plant or even on plant yields.

The yields recorded are presented in Table 2.

To be able to know the impact of the attack of the nematode on the yields of grain we have used the test of Spearman (Table 3).

DISCUSSION

The degrees of the studied plots infestation are heterogeneous and vary considerably from one town to another. At the level of the explored towns, degrees of infestations are divided into three levels:

1) first level: the town where the infestation is zero: Sebaine and Mahdia following the total absence of the cyst nematode *H. avenae*.

2) second level: towns where the infestation is low, does not exceed the threshold of harmfulness: Rahouia,Tiaret.

Common	Degree of infestation Cyst number/100 g soil	Degree of infestation Number of L2/1 g soil	Repair (Rivoal et al., 1980)
Dahmouni	15	39.39	heavily infested >10L2/ g soil
Frenda	13	29.72	heavily infested >10 The2/ g soil
Ain El Heddid	10	21.93	heavily infested >10L2/ g soil
Mechraasfa	8	11.41	heavily infested >10L2/ g soil
Sougueur	6	8.41	Moderately infested 10 L2 /g soil
Tiaret	3	6.41	Moderately infested 10 L2 /g soil
Rahouia	3	4.41	Moderately infested 10 L2 /g soil
Mahdia	0	0	free absence of L2 /1 G Soil
Sebaine	0	0	free absence of L2 /1 G Soil

Table 1. Repair of Commons explored depending on the degree of infestation
(number of cysts/100g and number of L2/g soil).

Common	Performance qx/ha				Average yield qx/ha
	Hard wheat	Soft wheat	Barley	Oats	
Dahmouni	15.75	13.69	15.00	12.00	14.97
Frenda	13.85	14.67	15.00	13.00	14.42
Ain El Heddid	13.33	13.65	14.32	13.00	13.64
MechraaSfa	23.20	19.33	20.00	21.00	23.43
Sougueur	12.86	14.01	15.63	8.00	13.44
Tiaret	16.84	16.30	16.00	12.00	16.30
Rahouia	23.18	18.00	22.00	13.00	22.87
Mahdia	11.67	14.83	15.79	10.00	13.58
Sebaine	12.89	14.44	14.87	10.00	13.46

Table 2. Yields recorded in the countryside

Species	Hard wheat	Soft wheat	Barley	Oats	Average
Correlation	-0.06	-0.26	-0.27	0.18	-0.16

Table 3. Correlation between the infestation and the performance.

3) third level: towns where the infestation is strong, far exceeding the threshold of harmfulness: Dahmouni, Frenda, Ain El Heddid, Mechraasfa and Sougueur.

The test of Spearman which has allowed us to study the correlation between the degree of infestation and the yields recorded of different cereals shows us the following.

The explored towns plots are all infested by *H. avenae* with a different degrees of infestation with

the exception of the plots in the town of Sebaine and Mahdia or the infestation is virtually non-existent (0 cysts /100 g of soil).

Yields in hard wheat

The correlation is negative between the degree of infestation and the performance (r = -0.06), yields are low when the degree of infestation is high (Fig. 1).

The region of Dahmouni is the most heavily infested with a degree of infestation of 15 cysts / 100g of soil, the performance in hard wheat is of 15.75 qx/ha. This degree has been reported by other authors such as: Smaha et al. (2009) and Labdelli et al. (2017). On the contrary, the town of Rahouia presents a higher performance with 23.18 qx/ha since the degree of infestation is low: 3 cysts /100 g of soil.

This could be due to the sensitivity of the hard wheat to the attack of the nematode *H. avenae*. Therefore, the hard wheat is a favorable host for this dreaded pest, as studied and confirmed by Sanchez et al. (1985).

Yields in common soft wheat

The yields of common soft wheat and the degrees of infestation are negatively correlated (r = -0.26). The performance decreases when the degree of infestation increases, therefore the heavy infestations significantly affect the yields of this type of culture.

For the region of Tiaret, a low level infestation (3 cysts / 100 g of soil) was recorded with a yield of 16.30 qx/ha.

The yields decrease if the infestation is higher, as it has been reported in the town of Frenda: the high infestation (13cysts/100g of soil) causes a decrease in the performance (14.67 qx/ha).

Barley yields

The correlation is negative (r = -0.27) between the degree of infestation and the performance. Therefore, the heavy infestations result in a decrease in performance.

For the region of Rahouia, with an infestation degree of 3 cysts/100g of soil, so the performance of the barley reached 22qx/ha, by contrast, the region of Ain el Heddid, with a degree of infestation of 10 cysts /100 g of soil, the performance becomes lower with 14.32 qx/ha. We can say that the Barley is a sensitive species to the attack of *H. avenae*.

Yields of oats

Unlike the other species of cereals, the correlation is positive with an infestation degree of r = 0.18, so the infestation of the nematode has not influenced the yields of oats largely.

Yields in the different municipalities are closer even if infestations become higher for example: in the town of Dahmouni with a degree of infestation of 15 cysts/100 g of soil, the performance is 12 qx/ha. Similarly, for the municipalities of Tiaret, Rahouia, the degree of infestation is 3 cysts/100g of soil for each; the yields are around the 12 qx/ha of the town of Dahmouni which is heavily infested with respectively 12 and 13 qx/ha.

In Northern Europe, Eastern oats are considered as favorable host for the development of the nematode, whereas in Southern Europe the wheats are the most damaged due to the coincidence of the development cycle of the wheats with the winter activity of the nematode in these regions (Rivoal & Riviere, 1989).

So we can say that the oats with positive correlation is a species tolerant to the attack of the nematode compared to wheat and barley that have registered declines in yields.

Unlike the three types of previous crops, oats is distinguished by its tolerance to this parasite.

CONCLUSIONS

Among the factors that reduce the yields of cereals in Algeria we can mention the cyst nematode of grain called *Heterodera avenae*, that occupies an important place prominently in view of the losses caused by this parasite. In the course of the present study undertaken in the region of Tiaret, the study of the impact of the infestation of *H. avenae* on yields has attracted the attention.

The prospecting of grain parcels in the region of Tiaret shows that the degrees of infestations registered by this nematode are variables. Apart from the regions of Mahdia and Sebaine where the nematode is completely absent, the regions of Dahmouni,
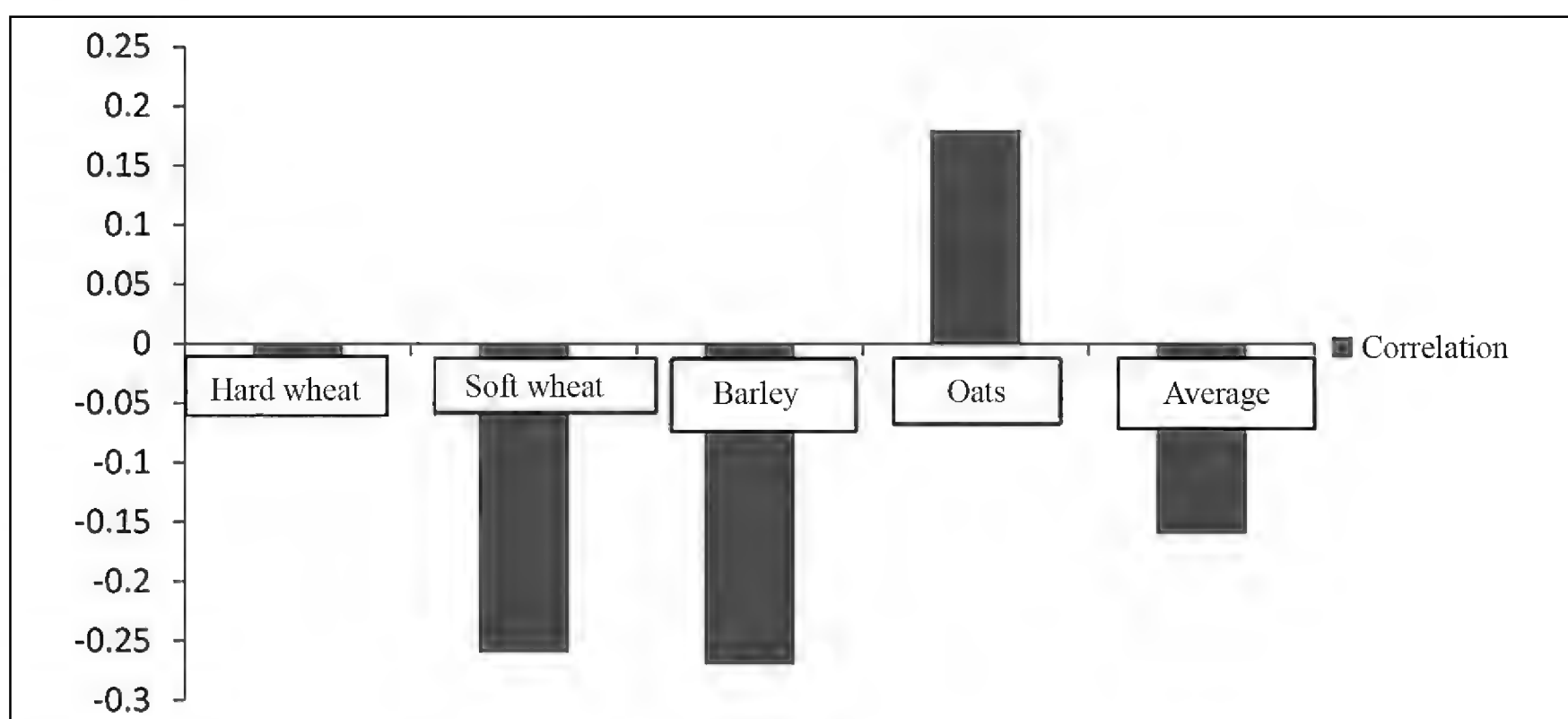
Figure 1. Histogram of the correlation between the infestation and yields.

Frenda, Ain El Heddid, MechraaSfa, Sougueur-Tiaret, Rahouia present high degrees of infestations. Yield reductions are reported on the productions of the common wheat and barley which showed a negative correlation with the infestations. On the contrary the oats are tolerant to the attack of the nematode compared to other species of cereal.

The fight against *H. avenae* remains difficult because of the high costs of chemicals. This directs the fight against this nematode toward preventive methods by the intervention of cultivation techniques (choice of resistant varieties) or the use of an intensive system with a fallow (year by year) or an adequate rotation.

These degrees of infestation are due mainly to the monofarming practiced.

The varietal resistance is currently one of the tracks the most interesting for the fight against bio-aggressors. The present study must be continued by taking into consideration the role of the Technical Itinerary: date of planting, the work of the soil and the crop rotation on the intensity of the infestations of the nematode in the soil.

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Influence of salinity soil on the sodium, potassium and calcium content of tomato *Lycopersicum esculentum* Mill. (Solanales Solanaceae) at 5-leaf stage

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ABSTRACT   The effect of salinity ($NaClCaCl_2$) on the evolution of mineral ions (Na^+, K^+ and Ca^{++}) and the variation of (Na^+/K^+ and Na^+/Ca^{++} ratios) in the roots of a Rio grande (Rg) tomato variety (*Lycopersicon esculentum* Mill., Solanales Solanaceae) at the 5-leaf stage was investigated. After germination of the seeds, the seedlings obtained are transferred to their environment to receive the nutritive solution. At 5 leaf stage, salt stress was applied with four concentrations (0, 100, 200 and 300 meq L-1 $NaClCaCl_2$) for 15 days. The results shows that salt stress increased Na^+ and decreased K+ content significantly in both cultivars. Increase of salinity resulted in an increase of ions Na^+ and Ca^{++} in the tomato root cultivated on the two substrates but a decrease of K+. The Na^+/K^+ and Na^+/Ca^{++} ratio in roots of both cultivars cultivated on the two substrates increased significantly when concentration of $NaClCaCl_2$ increased in the medium. The presence of calcium in the medium (irrigation solution and bentonite) favored an increase in the average amount of calcium ions in the roots of tomato grown in sand substrate mixed with bentonite compared to those cultivated in the sand substrate alone.

KEY WORDS   Bentonite; Na^+; K^+; Ca^{++}; Ratio; Root; Salinity; Tomato.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) (Solanales Solanaceae), one of the most important and widespread crops in the world, with total production and cultivated area estimated at 164 Mt and 4.76 Mha, respectively (Rodriguez-Ortega et al., 2019). Tomato is sensitive to moderate levels of soil salinity which is a major abiotic constraint that negatively affects the physiological and biochemical aspects of the plant, resulting in a reduction of its yield. In Algeria, tomato production is mainly located in areas with a semi-arid Mediterranean cli-

mate characterized by sandy soils and irrigation water loaded with abnormally high soluble salts. This situation causes a decrease in the yields of the tomato crop. Soil salinity impacts are not limited only to the environment but also extend to the economy (Allbed & Kumar, 2013). This also adversely affects plant growth, crop production, soil and water quality, eventually resulting in soil erosion and land degradation (Rhoades & Loveday, 1990; Zhu, 2001). In addition, it induces osmotic stress, physiological dryness and ionic imbalance, thereby disabling the plant's vital cellular functions (Gupta & Huang, 2014). This constraint affects

downward water availability, reduced respiration rate (Cramer et al., 2013), mineral salt distribution (Babu et al., 2012) and variations in turgor pressure (Shabala et al., 2012). Thus, plants trigger hormonal, physiological and biochemical mechanisms either by tolerating or resisting this constraint (Almeida et al., 2012). Salt stress influences growth through many facets of metabolism, such as nutrient uptake and distribution within the plant, alteration of photosynthesis (Taffouo et al., 2013), synthesis of proteins, accumulation of organic solutes, hormonal balance and water availability (Karuppasamy et al., 2014). In addition, the reduction in growth due to salinity is also attributable to ion toxicity and nutrient imbalance. This state causes not only an increase of accumulation of sodium (Na^+) and chloride (Cl^-) in plants, but also it affects antagonist of absorption of essential elements such as potassium (K+), calcium (Ca^{++}) and magnesium (Mg^{++}) with Na+ and nitrates (NO_3-) in contrast to Cl- (Zörb et al., 2005). The constituent cations of total soluble salts in soils are usually sodium (Na+), calcium (Ca^{++}), and magnesium (Mg^{++}) and the anions are chloride (Cl^-) and carbonate. However, Na+ dominates the cations and Cl- the anions in the majority of saline soils to the extent that NaCl comprises from 50-80 % of the total soluble salts (Rengasamy, 2006). Salt stress has reduced soil water potential leading to osmotic stress, induces ion imbalance in cells, especially lower concentrations of K^+, Ca++ and NO_3-, and causes ion (Na^+ and/or Cl^-) toxicity (Munns & Tester, 2008). Reduced Na+ loading into the xylem is one of the main mechanisms of salinity tolerance and it is often considered one of the most crucial features of restricting Na^+ accumulation in plant tissues (Munns & Tester, 2008). Thus, our research experiment investigated the effect of osmotic stress of salinity on the sodium, potassium and calcium contents, alterations in Na^+/K^+ and Na^+/Ca^{++} in root of tomato plants cultivated on the two different substrates at 5 leaves level.

MATERIAL AND METHODS

Plant materials and culture mode

Processing tomato (*Lycopersicon esculentum*) variety "Rio Grande" (Rg) was used as plant mate-rial for this experience. The crop is grown in two different types of substrate: sand (S) and sand mixed with 10% of bentonite (SB). The substrate is placed in plastic pots of 15 cm diameter and 17 cm height with a capacity of 1.3 kg.

Preparation of the culture substrate

The sand is washed beforehand with dilute hydrochloric acid, rinsed thoroughly with distilled water to remove the chlorides and dried in an oven at 105°C. Thus prepared, the sand constitutes a support of the plant, allows aeration of the roots and has the advantage of not fixing the ions (Demolon, 1968). After natural drying, the bentonite (clay of calcium origin) is crushed then mixed with the sand with a dose of 10%. The Sand-Bentonite mixture is carefully homogenized manually, then filled into the pots. Beforehand the bottom of the pots is lined with a layer of one cm thick gravel of 0.5 cm in diameter serving as drains. On this layer a gas strip is deposited to retain the sand. After germination, the plants was transplanted at the rate of one plant per pot, which constitutes an experimental unit repeated three times, followed by irrigation with the nutrient solution (Hoagland & Arnon, 1938). The saline stress was composed of four concentrations (Control, 100, 200 and 300 meq.L^{-1} of $NaClCaCl_2$), the control is sprinkled with the nutritive solution. Stress was applied on the 40th day of sowing, i.e. at the 5-leaf stage, then repeated at a frequency of three times a day until 55th day. A week later, the samples are taken to undergo mineral analysis (Na^+, K^+ and Ca^{++}) in the roots.

Mineral ions

Three plants per treatment are taken, after a passage in the oven at 80°C for 24 hours, the roots underwent an analysis of the various minerals. The assay is carried out in medium composed of H_2O_2 and $HClO_4$ from 0.5 g of ash placed in 100 ml flasks, to which 12.5 of hydrogen peroxide (H_2O_2) are added. After overnight rest, perchloric acid $HClO_4$ is added. Flasks are placed on a ramp and gently heated, then gradually cools. Then the distilled water is added to 100 ml. The assay is performed by a flame spectrophotometer. The percentage of each element is calculated by the following formula:

% (Na$^+$, K$^+$ and Ca^{++}) = (n/D)$*$(V/10^4)$*$W = in g.100 g^{-1} of DW.

n: a gluanometric number, D: dilution = 1, V: extraction volume = 100 ml, W: sample weight.

Statistical analysis

An experimental design of plots in complete random blocks was carried out using 3 plants per treatment. Three roots and three stems per plant were sampled for anatomical studies. Data were analyzed by ANOVA using the Newman-Keuls Least Significant Difference Test (LSD) for mean comparisons using a significance level of 5%.

RESULTS

Figure 1 shows the effect of soil salinity on the Na+ content of the roots. However, the low Na$^+$ values are recorded in the control plants with 1.78 mg.100 g^{-1} DW in the substrate S and (1.8 mg.100 g^{-1} DW) in the substrate SB; as soon as the salinity increases in the culture medium, the Na+ increases in the roots to give significant values to 300 meq of NaClCaCl$_2$ of 7.1 and 6.73 mg.100 g^{-1} of DW respectively in the S and SB (Table 1). Under the highest salinity (300 meq), the Na$^+$ reaches (74.85%) in the substrate S and (73.26%) in the SB compared to the control. The two-factor ANOVA reveals a significant effect of salinity, but not significant effect at P<0.05 of the substrate and salinity times substrate interaction (Table 1). The content of K$^+$ root decreases appreciably with increasing salt stress and in the two culture substrates (Fig. 1). The plants cultivated respectively in the substrates S and SB record values passing from 3.37 and 3.47 mg. 100 g^{-1} of DW in the control to 2.8 and 3.2 mg.100 g^{-1} of DW under 300 meq of NaClCaCl$_2$. The K$^+$ values in the roots decrease respectively to 300 meq of NaClCaCl$_2$ by 16.83% in the S and by 7.77% in the SB (Table 1). The two-factor analysis of variance reveals a not significant at P<0.05 of the treatment salinity, the substrate and the salinity-substrate interaction on the K+ ion (Table 1). The Na+/K+ ratio in tomato roots increases in saline environment. At 300 meq.L-1 of NaClCaCl$_2$, the roots cultivated in the substrate S record a rate of 79.36%, this ratio increases slightly in the roots raised in the substrate SB to reach 76.31% (Table 1) The analysis

of the variance with two factors reveals significant effect at P<0.05 of salinity, substrate and interaction salinity times substrate on the Na+/K+ ratio (Table 1).

Figure 2 shows that the calcium content of the roots decreases considerably under the effect of salt stress. The two substrates S and SB record respectively in the control plants 2.906 and 3.5 mg 100 g-1 of DW, these contents drop to 2.1, 0.92 and 0.52 mg 100 g-1 DW and 2.9, 1.55 and 1.42 mg 100 g-1 DW respectively at 100, 200 and 300 meq.L-1 of NaClCaCl$_2$ (Table 2). By comparing the two culture substrates, the calcium content of the roots decreased in the substrate S by 82.13% and 59.42% in the SB in the control compared to plants stressed with 300 meq.L-1 of NaClCaCl$_2$ (Table 2). Analysis of the variance shows a significant effect of the salinity and the substrate on Ca^{++}, but, no significant effect of the interaction salinity times substrate (Table 2). On the other hand, the selective Na$^+$/Ca^{++} ratio in the roots of the tomato increases under the effect of salt stress (Fig. 2). The lowest rates are recorded in the control roots with 0.611 and 0.517 respectively in the S and the SB. However, when the salinity increases in the culture medium, these rates increase at 13.6 and 4.73 successively in the substrate S and SB, these ratios increased by 95.5% in the S and by 89.06% in the SB (Table 2). The statistical study at P<0.05 showed a very significant effect of the salinity, the substrate and the interaction between the treatment substrate (Table 2).

DISCUSSION

Plants grown in a saline environment accumulate more sodium than potassium ions (Sudhir & Murthy, 2004). Generally, increased sodium accumulation causes potassium deficiency in plants stressed by salt, which indicates the existence of competitive effects between sodium and potassium ions (Maggio et al., 2007). Salt stress resulted in an increase in Na$^+$ ions and a decrease in K$^+$ ions in the roots. The increase in Na$^+$ in the roots decreases the osmotic potential, thus helping to maintain the difference in water potential between the roots and the medium necessary to obtain water from the saline solution. Na$^+$ competes with K$^+$ at entry sites and ultimately decreases the absorption of K$^+$ (Shabala et al., 2003). The ionic response of

the plant in saline medium is the reduction of the accumulation of K^+, by replacing K^+ by Na^+, which causes an ionic imbalance (Nedjimi & Daoud 2009). This competition can have significant negative effects on plant development and growth, where Na^+ levels often exceed those of K^+ (Tester & Davenport 2003). With other solutions, K^+ plays a major role in osmotic adjustment, the maintenance of turgor and, thus, the attenuation of the harmful effect of saline stress (Wang et al., 2013). However, figure 1 shows the effect of salinity on the Na^+/K^+ ratio, according to Alleva et al. (2006) and Akram et al. (2007), the high Na^+ concentration decreases the intracellular influx of K^+. Therefore, maintaining a low Na^+/K^+ ratio in cells is essential for plant tolerance to salt stress (Maathuis & Amtmann, 1999). In addition, there is a physiological relationship between ionic accumulation in the plant organ, the evolution of the selective relationship and the mechanisms of tolerance to salinity (Noble et al., 1984; Munns & Tester, 2008; Gupta & Huang, 2014). One of the mechanisms of

Substrate	Salinity	Na^+	K^+	Na^+/K^+
Sand	100 meq	3.233 [b]	3.233 [e]	0,999 [g]
Sand	200 meq	5.067 [c]	3.17 [e]	1.6 [h]
Sand	300 meq	7.067 [d]	2.8 [e]	2.52 [i]
Sand	Control	1.777 [a]	3.367 [e]	0.52 [f]
	Percent change	74.85%	16.83%	79.36 %
SWB	100 meq	4.067 [b]	3.387 [e]	0.743 [f]
SWB	200 meq	5.5 [c]	3.31 [e]	1.22 [j]
SWB	300 meq	6.733 [d]	3.203 [e]	2.027 [k]
SWB	Control	1.800 [a]	3.473 [e]	0.48 [f]
	Percent change	73.26%	7.77%	76.31%
Salinity	LSD at 5%	0.0000 ***	0.273 ns	0.0000 ***
Substrate	LSD at 5%	0.159 ns	0.195 ns	0.000042 ***
Salinity x Substrate	LSD at 5%	0.103 ns	0.887 ns	0.0404 *

Table 1. Two-factor ANOVA mixed randomly with alpha = 5%, Na^+, K^+ (mg 100 g-1 of DW) and Na^+/K^+ ratio in tomato roots grown on two substrates (S and SB) stressed with NaClCaCl2 at the 5-leaf stage; ns: not significant at $P < 0.05$; *, **, *** significant at $P < 0.05$, 0.01, and 0.001 respectively. Mean differences between substrate and stress is significant at $P < 0.05$, 0.01, and 0.001 respectively. Interaction substrate versus stress is significant at 0.05 and 0.01 respectively. Different letters are significantly different ($P < 0.05$).

tolerance to salinity is obtained with the absorption and accumulation of inorganic ions, mainly Na^+, K^+ and Cl^- (Munns & Tester, 2008). The regulation of ions is an essential factor in the salt tolerance mechanism of tomatoes (Shabala et al., 2010).

An excess of NaCl causes a Ca^{++} deficiency in the tomato roots. Plants subjected to saline stress see their calcium content decrease (Arshi et al., 2010). The decrease in calcium can be attributed to the competition of Na^+ and Ca^{++} ions for binding sites (Kaya & Higgs, 2003). Maintaining an ade-quate supply of calcium in soil saline solutions is an important factor in controlling the severity of specific ionic toxicities, especially in crops suscep-tible to damage by sodium and chloride (Maas, 1993; Grattan & Grieve, 1999). The amendment of the bentonite growing medium decreased the Na+ content in the roots. The presence of calcium im-proves the metabolism of other nutrients and regu-lates enzymatic and hormonal functions, while acting as a secondary messenger in the response to stress, it can reduce the excessive absorption of Na^+

Substrate***	Salinity***	Na^+	Ca^{++}	Na^+/Ca^{++}
Sand	100 meq	3.233 [b]	2.1 [f]	1.55 [k]
Sand	200 meq	5.067 [c]	0.92 [g]	5.56 [l]
Sand	300 meq	7.067 [d]	0.52 [g]	13.6 [m]
Sand	Control	1.777 [a]	2.91 [e]	0.611 [k]
	Percent change	74.85%	82.13%	95.5%
SB	100 meq	4.067 [b]	2,9 [i]	1,4 [k]
SB	200 meq	5.5 [c]	1.55 [j]	3.55 [ln]
SB	300 meq	6.733 [d]	1.42 [j]	4.73 [lo]
SB	Control	1.800 [a]	3.5 [h]	0.517 [k]
	Percent change	73.26%	59.42%	89.06%
Salinity	LSD at 5%	0.0000 ***	0.00000 ***	0.00000 ***
Substrate	LSD at 5%	0.159 ns	0.00000 ***	0.00000 ***
Salinity x Substrate	LSD at 5%	0.103 ns	0.715 ns	0.00000 ***

Table 2. Two-factor ANOVA mixed randomly with alpha = 5%, Na^+, Ca^{++} (mg 100 g-1 of DW) and Na^+/Ca^{++} ratio in tomato roots grown on two substrates (S and SB) stressed with NaClCaCl2 at the 5-leaf stage; ns: interaction substrat vs stress non significative. *: mean differences for all effects were significant at P < 0.05, 0.01, and 0.001 respectively. *, **, ***: differences between substrate is significant at P < 0.05, 0.01, 0.001 respectively. The same letters are not significant at P< 0.05.
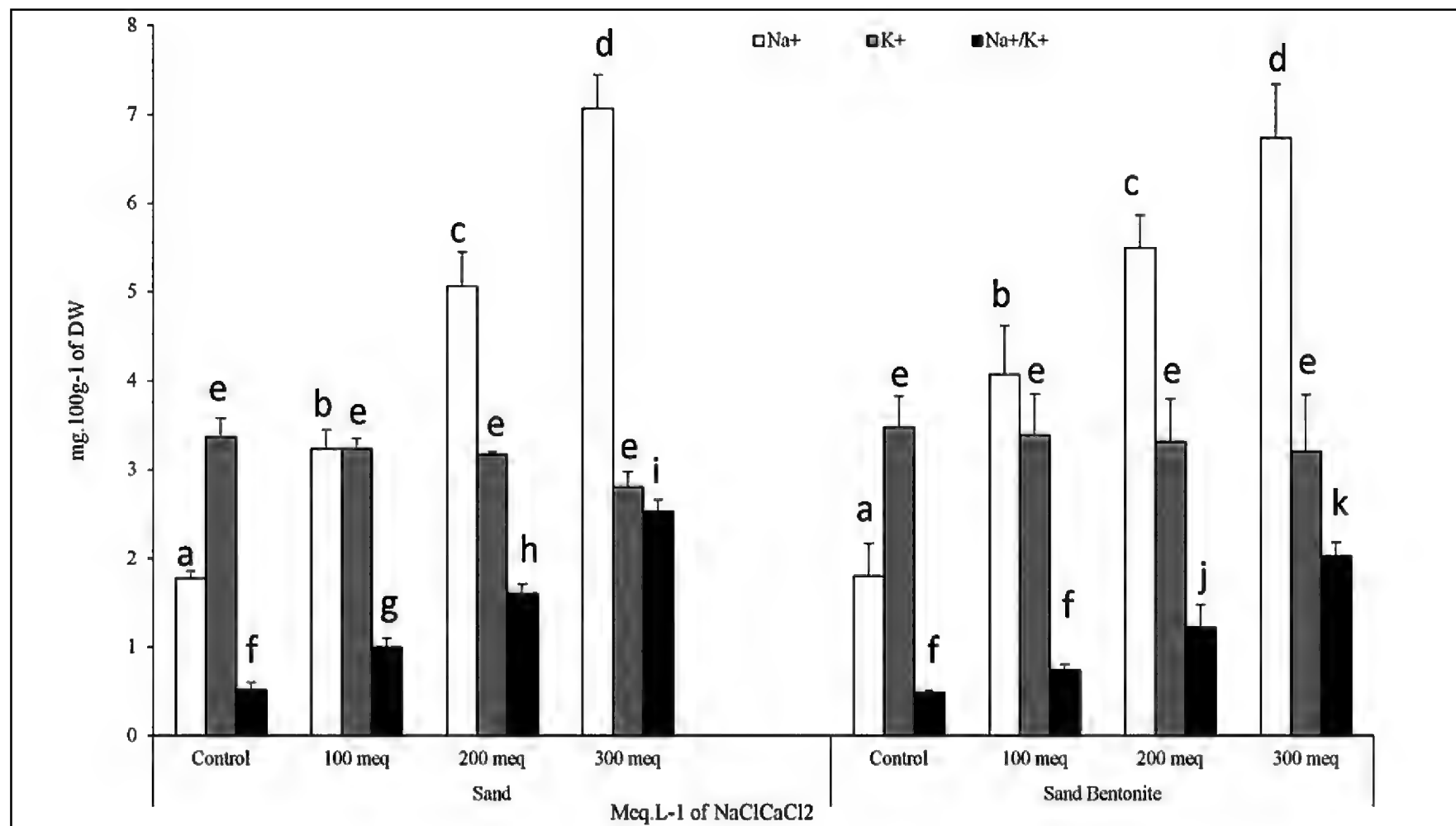
Figure 1. Na$^+$, K$^+$ (mg.100 g-1 of DW) and Na$^+$/K$^+$ ratio in the tomato (*Lycopersicon esculentum*) plants roots grown on two substrates (sand and sand mixed with bentonite) under NaClCaCl$_2$ stress at the 5-leaf stage. Data represent the mean of tree replication and error bars indicate SD. Different letters are significantly different (p <0.05). *, **: Differences between substrate is significant at P<0.05, and 0.01 respectively, the same letters are not significant at P< 0.05.
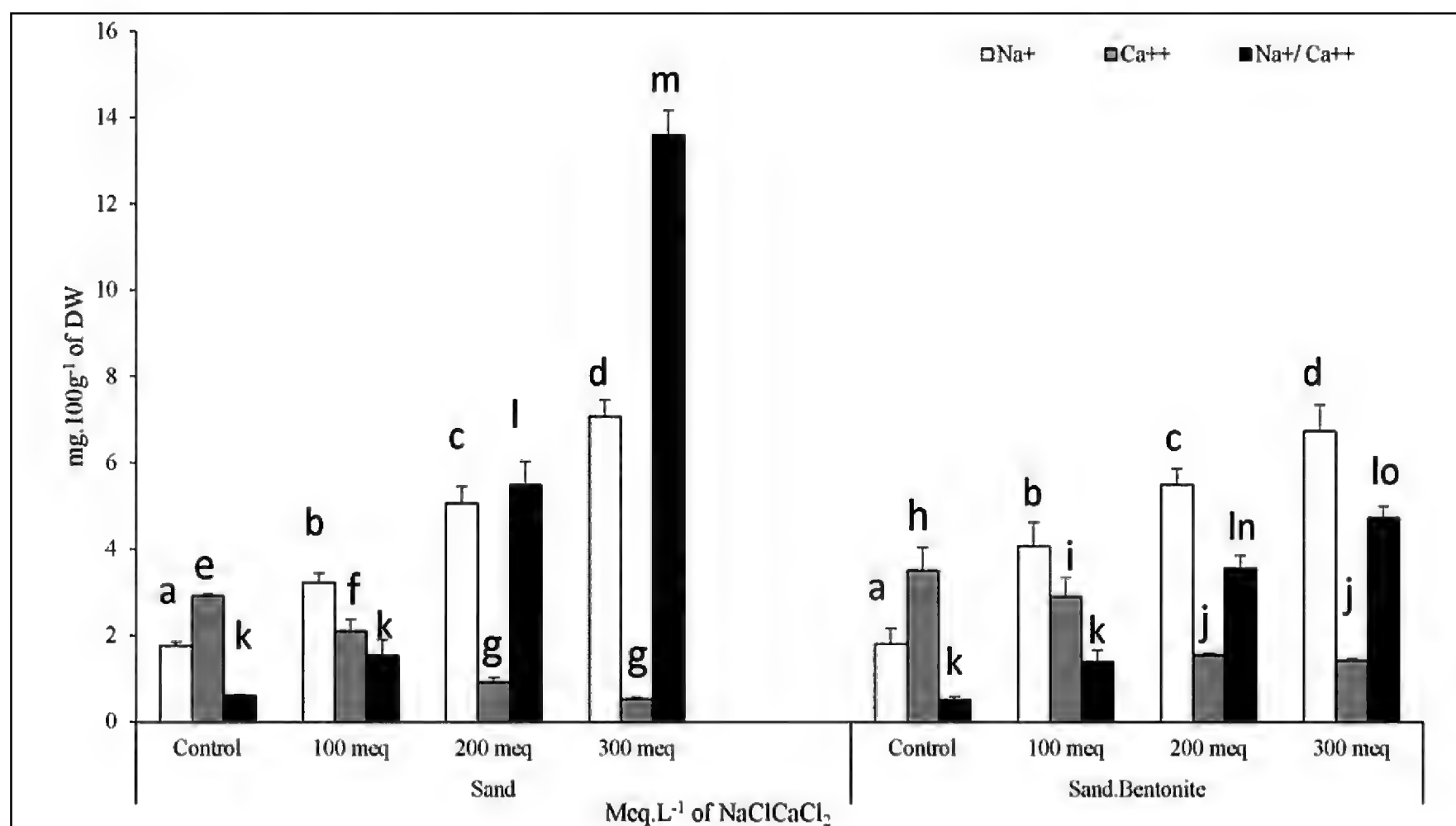
Figure 2. Na$^+$, Ca^{++} (mg.100 g^{-1} of DW) and Na$^+$/Ca^{++} in the tomato (*Lycopersicon esculentum*) plants roots grown on two substrates (S and SB) under NaCl+CaCl$_2$ stress at the 5-leaf stage. Data represent the mean of tree replication and error bars indicate standard deviation (SD). Different letters are significantly different (p <0.05). The same letters are not significant at P< 0.05.

from plants cultivated in a saline environment (White & Broadley, 2003). The Na^+/Ca^{++} ratio decreased in bentonite compared to sand alone under the effect of high salt concentrations. Calcium bentonite has helped tomato roots adapt to salt stress by reducing the toxic effects of NaCl and its contribution to sandy soils may have reduced the harmful effects of salinity.

CONCLUSIONS

The amendment of the sand with bentonite made it possible to see a correct ionic supply of the plant by the roots placed in a control medium, and completely eliminates the inhibitory effects of the salt on growth: it is therefore the salt of the medium which disturbs the mineral diet of the plant. We deduce that bentonite makes more nutrients and water available to the culture than the sandy substrate because of the leaching linked to the permeability of the sand which retains less nutrients than the mixture bentonized sand. In general, the tomato variety reacts to $NaClCaCl_2$, and it is the concentration of 300 meq l^{-1} which strongly influences all of the two substrates. Adding bentonite to the sand has reduced the effect of the salt.

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New data on the seasonality of *Flabellina affinis* (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) (Gastropoda Nudibranchia) in the Ionian Sea, Central Mediterranean

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ABSTRACT   *Flabellina affinis* (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) are two common nudibranchs in the Mediterranean Sea. However, there are only a few studies on their seasonality which reported these species principally in summer and in well-lit shallow areas. Instead, through the present study carried out throughout three years (from 2017 to 2019) in three areas sited along the Ionian coast of Sicily (Italy), it has been observed that: 1) both species may be present in any season of the year with a high number of specimens; 2) *F. affinis* in the study areas is more competitive than *C. peregrina*; 3) both species showed a less photophilous lifestyle than that usually reported in literature, since in this study both species were found in a deeper bathymetric range; 4) *F. affinis* and *C. peregrina* could be considered warm-water species and their strong presence in cold seasons might be used as an indicator of the increase in the seawater temperature of the Mediterranean Sea.

KEY WORDS   *Cratena peregrina*; *Flabellina affinis*; Ionian Sea; Nudibranchia; seasonality.

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INTRODUCTION

Flabellina affinis (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) are two nudibranchs of the family Flabellinidae and Facelinidae, respectively. *Flabellina affinis* (Fig. 1) is a common species, distributed in the Mediterranean Sea, along the Atlantic African coasts and Canary Islands (Trainito & Doneddu, 2014; Ballesteros et al., 2012–2020). This species is irregularly pink coloured with from six to nine ceratal clusters on each side of the back. Each ceratal cluster originates from a peduncle, which divides in several branches. Rhinophores have from 17 to 36 annulations and oral tentacles are shorter than cerata and almost long as rhinophores (Schulze & Wägele, 1998). The

eggs of this species usually are produced in a tangle with a pink-violet colouring (Figs. 2, 3) (Trainito & Doneddu, 2014).

Also *C. peregrina* (Fig. 5) is a common species, distributed in the Mediterranean and along the Atlantic coasts of Iberian Peninsula and in Canary Islands (Trainito & Doneddu, 2014). *C. peregrina* has a white transparent body with cerata arranged in 9 groups on each side of the back. The cerata can be long as the rhinophores, which are smooth and cylindrical. The oral tentacles are twice as long as the rhinophores. From the base of oral tentacles to the base of the rhinophores, on each side of the head there is an evident orange spot (Schmekel & Portmann, 1982). The eggs of *C. peregrina* are produced in a tangle which can

be whitish-pink coloured (Figs. 6, 7) (Trainito & Doneddu, 2014).

Flabellina affinis is a photophilous species, reported on different habitats: among photophilous seaweeds in rocky coasts, in the coralligenous biocenosis, in *Zostera* meadows, on sandy bottoms, on encrusting sponges, rarely on *Posidonia oceanica* (Linnaeus) Delile, 1813 and frequently on hydrozoans [particularly on *Eudendrium ramosum* (Linnaeus, 1758) and *Eudendrium racemosum* (Cavolini, 1785)]. Sometimes, *F. affinis* can be also found in dark cavities and on deep bottoms, even though this species was reported in lighted areas near the surface (Ros, 1975; Barletta & Melone, 1976). Concerning its seasonality, according to Ros (1975), *F. affinis* is abundant during summer along with other species such as *C. peregrina* and *Dondice banyulensis* Portmann & Sandmeier, 1960. Moreover, Ballesteros (1980), in his faunistic study on nudibranchs and sacoglossan of Catalan coasts, reported that *F. affinis* is a prevalently summer species and its principal bathymetric range is between 5–10 m. According to Betti (2011), in the area of Conero (Adriatic Sea), *F. affinis*, with *C. peregrina*, is a species very frequent during summer, which disappears in autumn and reappears the next summer. *F. affinis* usually feeds on some species of hydrozoans such as *Eudendrium glomeratum* Picard, 1952, *E. racemosum*, *E. ramosum* (Haefelfinger, 1960; Ros, 1975; Barletta & Melone, 1976; Schmekel & Portmann, 1982; Hirano & Thompson, 1990; McDonald & Nybakken, 1997; Betti, 2011, Trainito & Doneddu, 2014).

Similarly, *C. peregrina* is a photophilous species which was reported on different types of substrates: on rocky coasts, among seaweeds at 20 m depth, in *P. oceanica* and *Zostera* meadows, at caves' entrance, on sandy bottoms, rarely in coralligenous biocenosis and frequently on *Eudendrium* spp. (particularly on *E. ramosum* and *E. racemosum* colonies in well-lit vertical walls) (Ros, 1975; Barletta & Melone, 1976). Ballesteros (1980) reported that *C. peregrina* is particularly distributed in a range of depth of 0–10 m. According to Betti (2011), in the area of Conero this species is very abundant and particularly frequent on well-lit rocky bottoms, also in shallower waters, on *Eudendrium* spp. colonies and it spawns at the beginning of summer. Furthermore, in this area, *C. peregrina* is
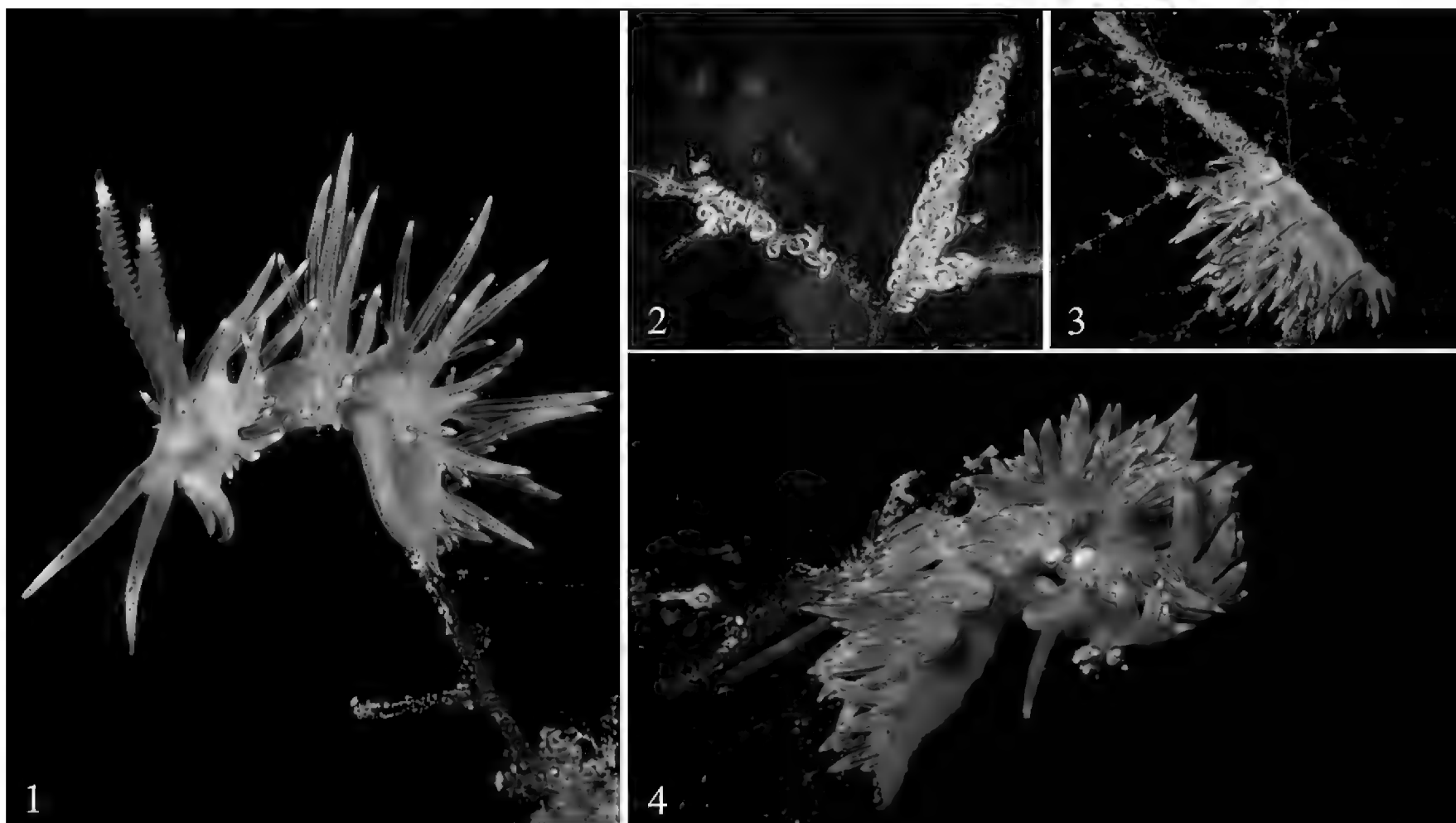
Figures 1–4: *Flabellina affinis* from the central-eastern coasts of Sicily. Fig. 1: lateral view of a *F. affinis* specimen. Fig. 2: *F. affinis* eggs on *Eudendrium* sp. Fig. 3: a *F. affinis* specimen during spawning on *Eudendrium* sp. Fig. 4: two specimens during breeding (photos A. Lombardo).

present from the late spring to the beginning of autumn (Betti, 2011).

Cratena peregrina usually feeds on different hydrozoans: *Eudendrium rameum* (Pallas, 1766), *E. racemosum*, *E. ramosum*, *Pennaria disticha* Goldfuss, 1820, *Pennaria* spp. e *Tubularia* spp. (Haefelfinger, 1961; Ros, 1975; Barletta & Melone, 1976; Schmekel & Portmann, 1982; McDonald & Nybakken, 1997; Betti, 2011; Trainito & Doneddu, 2014).

Although *F. affinis* and *C. peregrina* are very common and easily observable species in the Mediterranean Sea, data on their seasonality are scarce, particularly in the Ionian Sea. Indeed, information on the seasonality of these two species came only from the Catalan coasts of Spain (Ros, 1975) and the Adriatic Sea (Betti, 2011). The aim of the present research is to gain knowledge on seasonality of *F. affinis* and *C. peregrina* along the central-eastern coast of Sicily (Ionian Sea).

MATERIAL AND METHODS

This study has been carried out throughout three years (from 2017 to 2019) in different sites located along the central-eastern coast of Sicily (Italy) (Figs. 9, 10). The sites were selected basing on different environmental conditions. Two sites, Ognina (37°31'50.4"N – 15°07'10.8"E) and Bellatrix (37°32'03.2"N – 15°07'35.2"E) are both located in the municipality of Catania and are the most anthrophized sites among the study areas. Since they have the same environmental conditions and are situated close to each other, were considered as a single site, listed from now on as "Catania". Two sites, "Scalo Pennisi" (37°38'23.2"N – 15°11'04.6"E) and "Acque Fredde" (37°38'15.7"N – 15°10'52.1"E), are both situated in the hamlet of Santa Tecla, in the municipality of Acireale. These sites are the least polluted among the study areas. Therefore, since they have similar environmental conditions and are close to each other, they have been considered as a single site and listed from now on as "Santa Tecla". Finally, the last study station was Santa Maria La Scala (37°36'46.5" N – 15°10'31.4"E), in the municipality of Acireale, which has intermediate conditions between those of Catania and Santa Tecla and, thus, was considered
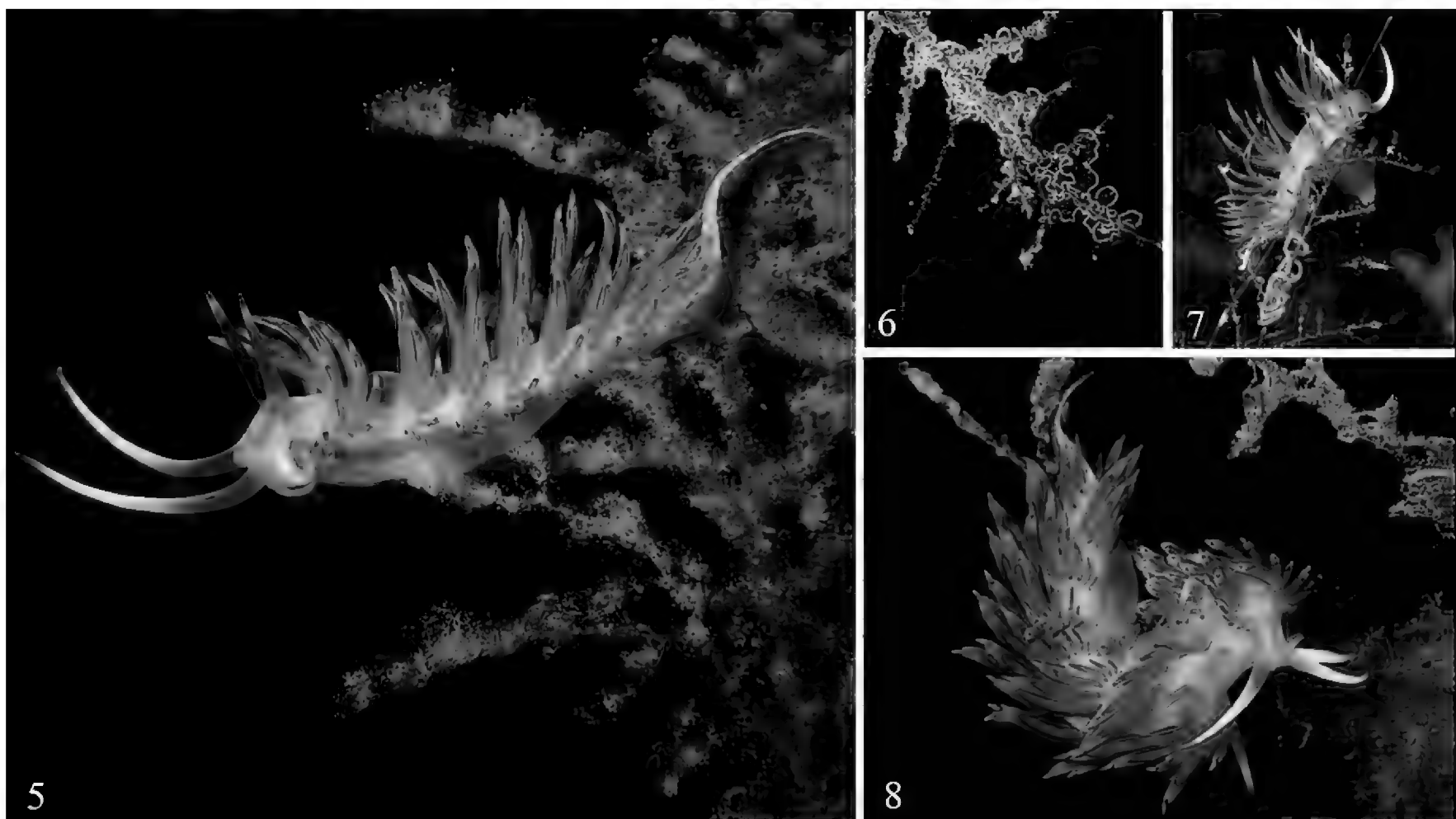
Figures 5–8: *Cratena peregrina* from central-eastern coasts of Sicily. Fig. 5: lateral view of a *C. peregrina* specimen. Fig. 6: *C. peregrina* eggs on *Eudendrium* sp. Fig. 7: a *C. peregrina* specimen with eggs on *Eudendrium* sp. Fig. 8: two specimens before breeding (photos A. Lombardo).
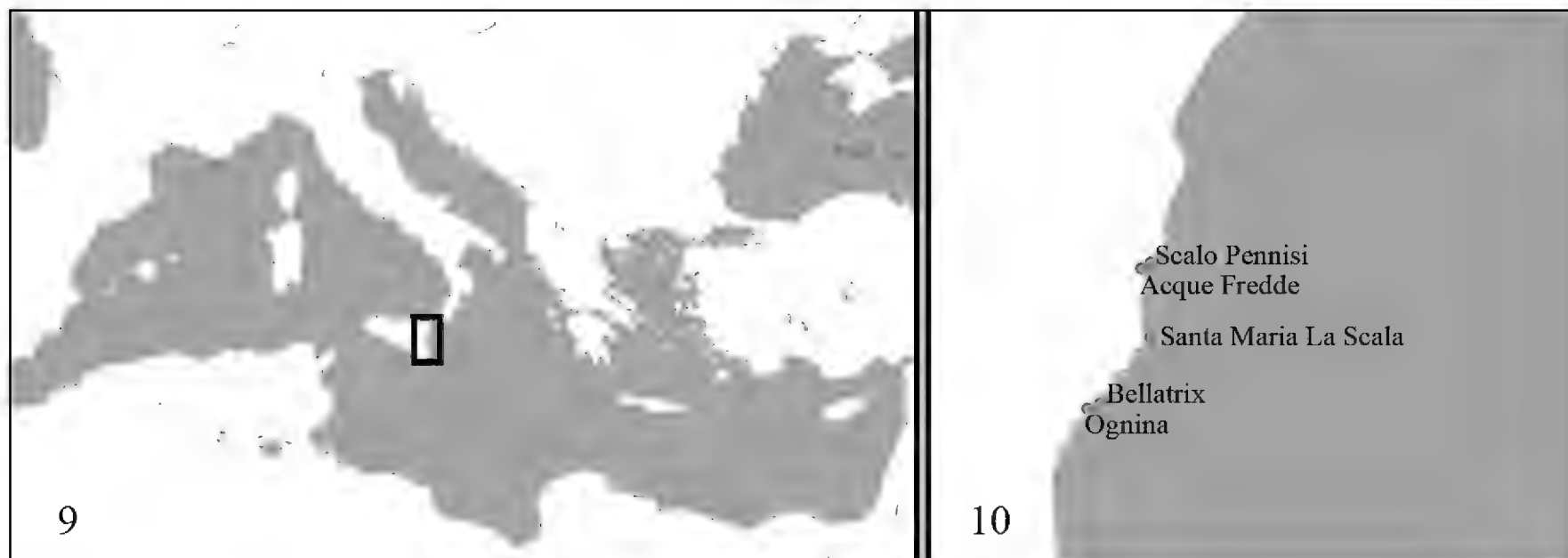
Figures 9, 10: Study area. Fig. 9: eastern coast of Sicily. Fig. 10: study sites within the central-eastern coast of Sicily.

individually. Both Santa Maria La Scala and Santa Tecla present several springs due to the flow of freshwater from the Etna to the sea (Ferrara, 1977; Catra et al., 2006). Data were collected through underwater visual census with scuba diving. In particular, a total of 224 dives has been realized: 74 in Santa Maria La Scala, 83 in Catania (Bellatrix and Ognina), 67 in Santa Tecla (Scalo Pennisi and Acque Fredde). Each scuba dive (in a range of depth of about 0–45 m, according to the seabed geomorphology) was conducted all year round (marine-weather conditions permitting), twice a week, during daylight, between 9–11:30 am. For each site, the same path was followed and *F. affinis* and *C. peregrina* specimens were photographed with an Olympus TG–4 underwater camera and counted in situ. For each station and for each species, the average minimum depths and the average maximum depths, in which the specimens were encountered, have been obtained.

The seasons were considered as follows: winter (December, January, February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November). Through data collection, the demographic trend of both species was assessed. In addition, for each station, specific density was evaluated as mean number of individuals/diving number per site. The experimental design adopted was completely randomized and replicated four times. Data were subjected to analysis of variance (ANOVA). Mean comparisons were performed according to Tukey Minimal Difference (MDS).

RESULTS

Catania (Figs. 11, 12)

Catania was the site with the highest average number of *F. affinis* specimens, throughout the three years of study. During 2017, winter represented the season with the maximum average number of found specimens (27.83). The other seasons presented the same trend (f), which was different than the winter trend. Indeed, between winter and the other seasons, there was a decrease of -78.87% (p<0.01) in the average number of specimens. During 2018, each season presented a different trend. In fact, winter had the highest average number of specimens (36), while autumn was the season with the lowest average number of specimens (5). Between these seasons there was a reduction of -86.11% (p<0.01). In spring, there was a reduction in the average number of specimens, while in summer there was a slightly increase (21.66). During 2019, there was the most stable seasonal trend than the previous years. During this year, winter was the season with the highest average number of specimens (19.66), while autumn was the season with the lowest average number of specimens (8.66). Between winter and autumn, there was a decrease of -55.95% (p<0.01). Spring and summer presented the same trend (e). In Catania, *F. affinis* specimens were found at an average depth-range of 16.5–32.1 m. Concerning the breeding and spawning activities (Figs. 3, 4), during 2017 they were observed in any season of the year. In 2018, the eggs were found all year round, while the breeding activity was

documented in winter, spring and summer. In 2019, the eggs were seen throughout all year, while the breeding activity was observed in summer and autumn.

Regarding *C. peregrina*, in this site the seasonal trends were all different in each year of study, with an almost stable presence of a low average number of individuals. During both 2017 and 2018, a rather steady and similar population trend has been observed, with the exception of spring 2017 in which the lowest average number of specimens has been found (1.00). Indeed, between winter and spring 2017, there was a decrease of -75.96% (p<0.01) of the average number of specimens. During 2019, contrary to the previous years, winter presented the least average number of individuals. In this year, spring and summer showed a similar trend with a slightly higher average number of specimens. In 2019, autumn had the highest average

number of individuals among all year of study (9.66). Between summer and autumn, there was an increase of 190% (p<0.01). In the site of Catania, *C. peregrina* specimens were averagely found in the bathymetric range of 9.7–25.8 m. With regard to *C. peregrina* eggs (Fig. 6–7), they were found during 2017 in summer and autumn, in 2018 they were observed in winter, summer and autumn, in 2019 they were documented in all seasons. For this species, the breeding activity (Fig. 8) was seen only in the winter 2018.

Santa Maria La Scala (Figs. 13, 14)

Santa Maria La Scala was the site with an average number of *F. affinis* specimens which was intermediate between the values observed for Catania and Santa Tecla. During 2017, winter was the season with the highest average number of
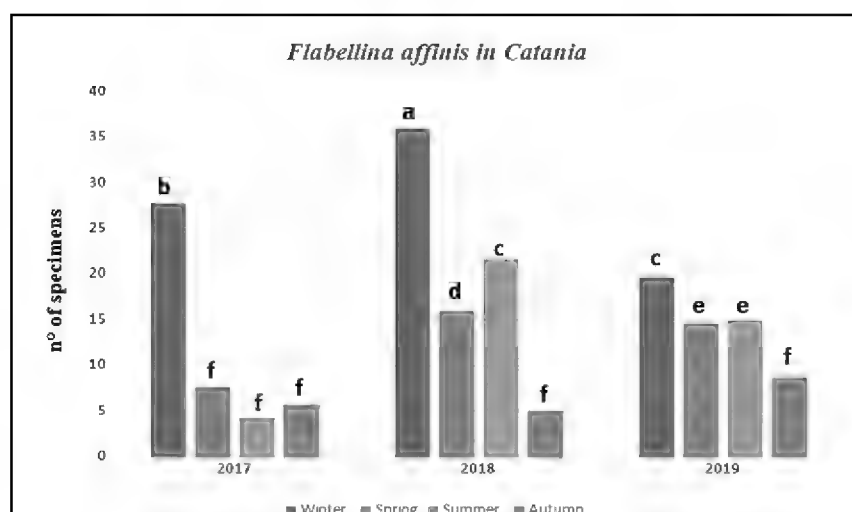
Figure 11. Oscillations in the average number of *F. affinis* specimens found in Catania during the three years' trial. Different letters indicate differences at p<0.01.
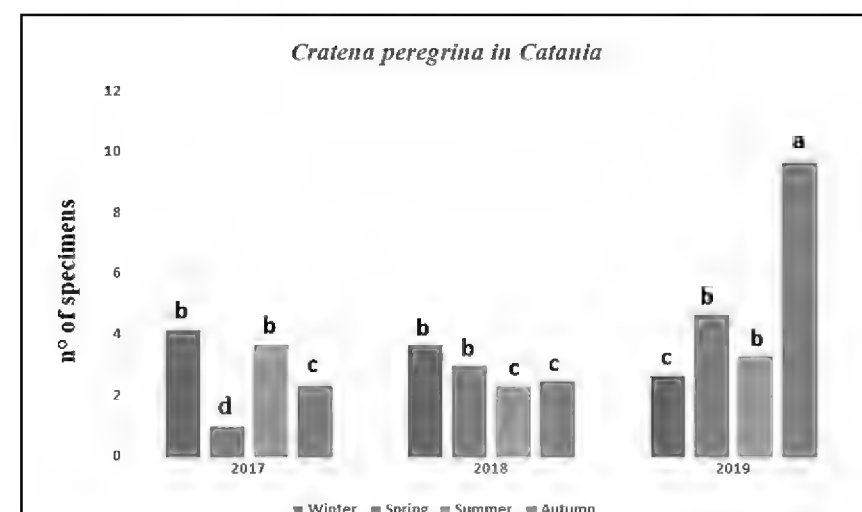
Figure 12. Oscillations in the average number of *C. peregrina* specimens found in Catania during the three years' trial. Different letters indicate differences at p<0.01.
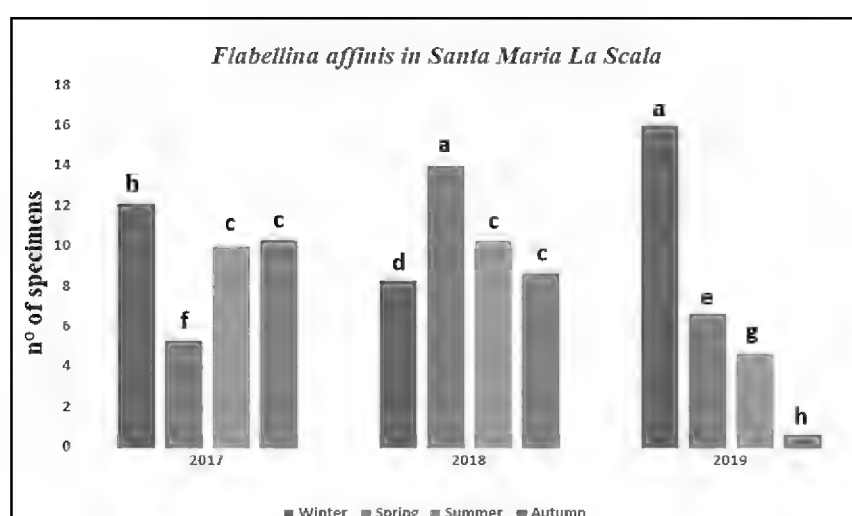
Figure 13. Oscillations in the average number of *F. affinis* specimens found in Santa Maria La Scala during the three years' trial. Different letters indicate differences at p<0.01.
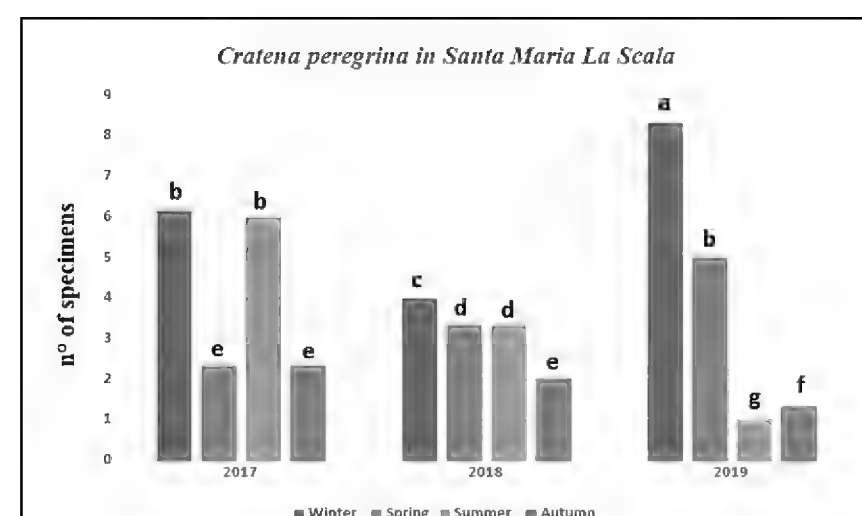
Figure 14. Oscillations in the average number of *C. peregrina* specimens found in Santa Maria La Scala during the three years' trial. Different letters indicate differences at p<0.01.

found individuals (12.17). Summer and autumn had the same trend, similar to the winter trend. Instead, spring was the season with the lowest average number of specimens (5.33). Between winter and spring there was a decrease of -56.20% (p<0.01). Oppositely, during 2018, the season with the greatest average number of individuals was spring (14.00), while that with the lowest average number of specimens was winter (8.33). Between winter and spring there was an increase of 68.06% (p<0.01). As in 2017, also in 2018, summer and autumn had the same trend. Instead, during 2019, a sharp decline of -95.81% in the average number of *F. affinis* specimens occurred between winter and autumn. In Santa Maria La Scala, *F. affinis* specimens were averagely found in a bathymetric range of 19.6–34.8 m. In this study area, the breeding and spawning activities were documented in all seasons during 2017. In 2018, the eggs were found all year round and the breeding activity was observed in winter and spring. During 2019, the eggs were found in spring, summer and autumn and no breeding activity was observed.

In Santa Maria La Scala, during each year of study, the seasonal trends of *C. peregrina* were different. In 2017, two main trends were observed: one identical for winter and summer (b) and another equal for spring and autumn (e). Each passage from trend (b) to trend (e) was signed by a decrease of -62.17% (p<0.01). During 2018, there was a more stable trend of the average number of *C. peregrina* specimens with a decrease from winter to autumn of -50% (p<0.01). During 2019, there was a winter peak (8.33) and sharp decline in summer (1.00) of the average number of specimens. From winter to summer, a decrease of -87.99 % (p<0.01) was observed. In this site, on average, *C. peregrina* specimens were observed in a depth-range of 12.4–33.5 m. Furthermore, *C. peregrina* eggs were found in all seasons during both 2017 and 2018, while throughout 2019 they were seen in winter, spring and summer.

Santa Tecla (Figs. 15, 16)

Santa Tecla was the study area with the lowest average number of *F. affinis* specimens (4.86). During 2017, the seasons with the highest values of the average number of specimens were summer and autumn (~7.40), while the season with the lowest

average number of found specimens was spring (1.00). Between spring and summer-autumn there was an increase of 641% (p<0.01) in the average number of *F. affinis* specimens. During 2018, there was a more stable seasonal trend and only in spring there was a slight decrease. Instead, during 2019, almost all seasons, except for winter and autumn, presented different trends. Between winter and summer, the season with the greatest average number of specimens, there was an increase of 200.42% (p<0.01). In Santa Tecla, on average, *F. affinis* specimens were found in the bathymetric range of 16.6–26.1 m. In this site, during 2017, *F. affinis* eggs were found in spring and autumn. In 2018, the eggs were observed throughout all seasons, while the breeding activity was documented only in winter. During 2019, the eggs were found in spring, summer and autumn.

In Santa Tecla, during each year of study, a different seasonal trend of *C. peregrina* has been observed. During 2017, there was an identical trend for winter and summer (d), and two different trends for spring and autumn. Between winter and spring there was a decrease of -47.31% (p<0.01), while between summer and autumn there was a reduction of -66.66% (p<0.01) in the average number of specimens. During 2018, each season presented a different trend of the average number of *C. peregrina* individuals. After a decrease of -91.75% (p<0.01) occurred between winter and spring, the season with the lowest average number of found specimens in this site, there was an increase of 2830% (p<0.01) in autumn, the season with the highest average number of found specimens. Instead, during 2019, there were two distinct trends: one equal for winter and summer (e) and another identical for spring and autumn (b). Between these trends there was an increase of 154.62% (p<0.01) in the average number of specimens. In Santa Tecla, *C. peregrina* specimens were averagely found in the bathymetric range of 11.4–19.6 m. Regarding *C. peregrina* eggs, they were found during 2017 only in summer, during 2018 in summer and autumn and in all seasons of 2019.

DISCUSSION

In the present study the seasonality of two common eolidacean nudibranchs, *F. affinis* and *C.*
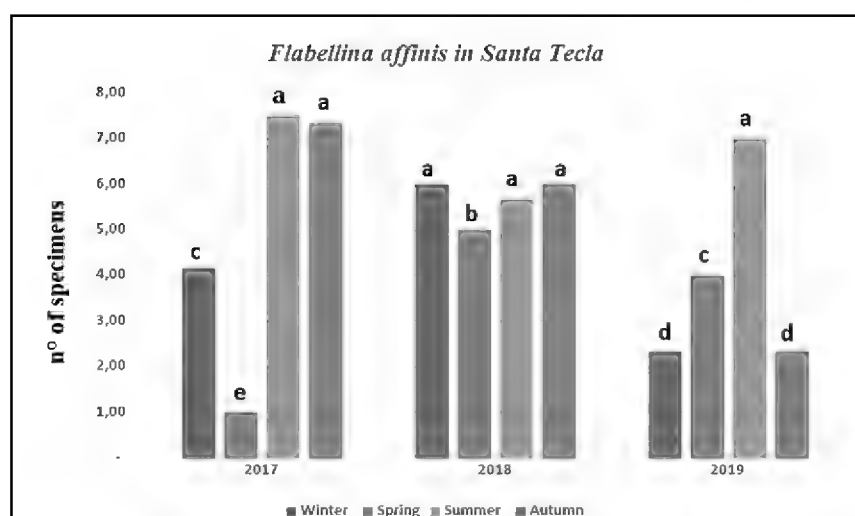
Figure 15. Oscillations in the average number of *F. affinis* specimens found in Santa Tecla during the three years' trial. Different letters indicate differences at p<0.01.
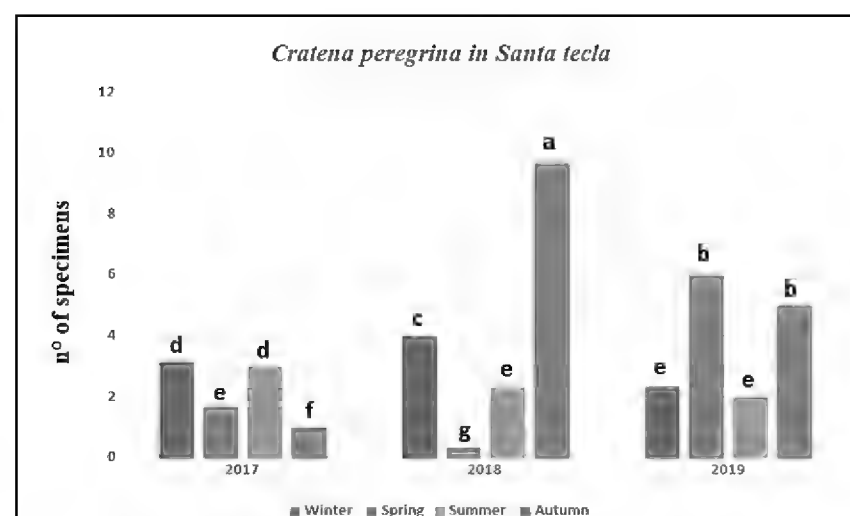
Figure 16. Oscillations in the average number of *C. peregrina* specimens found in Santa Tecla during the three years' trial. Different letters indicate differences at p<0.01.

peregrina, has been investigated throughout three years (from 2017 to 2019), in three areas along the central-eastern coast of Sicily. During the data collection, it has been observed that these species were present in all seasons and years. Probably, the presence of *F. affinis* and *C. peregrina* all year round could be related to the presence, in all study areas, of *E. racemosum* and *E. glomeratum*, which alternate during the year. In fact, according to Bavestrello et al. (2006), *E. racemosum* colonies are active from April and are fertile during the summer, degenerating in the autumn, while *E. glomeratum* is a winter species that appears in mid-October and decreases until April (Boero, 1984). Consequently, since these hydrozoans constitute the diet of both *F. affinis* and *C. peregrina*, these nudibranchs may have the food resources available all year round. Moreover, the presence in some seasons of a higher or lower number of specimens of these species, could depend on a major or minor larval recruitment. Indeed, according to Clark (1975), the sudden appearances of population often are due to the arrival of large numbers of allochtnous-produced larvae, that is related to critical temperatures that stimulate settling and metamorphosis. Furthermore, the decrease in the number of specimens of these species could also depend on the presence of predators. In particular, during 2019 in the site of Santa Maria La Scala, we observed a strong decrease in the populations of both *F. affinis* and *C. peregrina*, concomitantly with the increase in the number of specimens of another nudibranch species, *D. banyulensis* in the same bathymetric range of the examined species (personal observations). Indeed, *D. banyulensis* is a predator of other nudibranchs

and hydrozoans (Betti, 2011). Consequently, *F. affinis* and *C. peregrina* populations in this station during 2019 may have been decimated by competition and predation caused by *D. banyulensis*.

Generally, in all study areas, the number of *F. affinis* specimens was greater than the number of *C. peregrina* specimens. There are two main hypotheses that could explicate this trend: in the study sites *F. affinis* might be a more competitive species than *C. peregrina*, otherwise the larval recruitment of *F. affinis* is higher than the recruitment of *C. peregrina*. Furthermore, it has been observed that the number of *F. affinis* specimens, increase southwards and, thus, Catania was the study area with the highest average number of individuals. This event could depend on the fact that allochtnous-produced larvae of *F. affinis* might be carried through the powerful Algerian Current (Di Silvestro et al., 2010), coming from the southern Mediterranean and thus, Catania, among the studied areas, is the site with the greatest recruitment. The same behaviour was not observed for *C. peregrina*, whose average number of specimens was almost identical for each site. However, we believe that generally the allochtnous-produced larvae of both species could be transported by the modified Atlantic water (MAW) flow from the western Mediterranean Sea otherwise through the Levantine intermediate water (LIW) flow form the eastern Mediterranean Sea (Pirkenseer, 2013). Through data collection, it has been seen that the *F. affinis* depth-range begins deeper than the bathymetric range of *C. peregrina*, which it has also been observed in shallower waters. These data are different than those reported in the

literature cited above (Ros, 1975; Barletta & Melone, 1976; Ballesteros, 1980; Betti, 2011), which stated that the presence of these nudibranchs is principally in shallower waters, in well-lit areas. Furthermore, differently from Betti (2011) who reported the presence of both species particularly in summer in the area of Conero (Adriatic Sea), we noticed that both species in the investigated areas can be present with peaks in any seasons of the year. This different trend could be explained by two hypotheses. On one hand, since Sicily belongs to the southern sectors of the Mediterranean Sea, the seawater temperature is usually warmer than the Adriatic Sea. Indeed, in the Ionian Sea the seawater temperature fluctuates between 14–23°C, while in the Adriatic Sea the seawater temperature ranges from 11°C to 23°C (Lejeusne et al., 2009). Therefore, since in the cited literature (Ros, 1975; Barletta & Melone, 1976; Ballesteros, 1980; Betti, 2011) *F. affinis* and *C. peregrina* are present principally during summer, they seem to be warm-water species. Consequently, in the Ionian coast of Sicily these species could settle in any period of the year, while in the Adriatic Sea the settlement might occur in summer, because of the colder winter seawater temperature. On the other hand, in the last decades, we are experiencing a general increase in the seawater temperature of the Mediterranean Sea, caused by the global warming (Bianchi et al., 2018). In fact, a 30-year data set (1974–2005) from the Spanish Catalan coast, revealed a clear warming trend at four different depths from the surface to 80 m, with an impressive warming of 1.4 °C at 20 m (Lejeusne et al., 2009). Also in the Aegean Sea, satellite sea surface temperature data from the last 20 years showed a significant 1 °C warming trend (Lejeusne et al., 2009). Therefore, since *F. affinis* and *C. peregrina* are probably warm-water species, the presence of them in any season of the year could be an indirect indicator of the warming of the Mediterranean Sea.

In conclusion, through the present study new data on the seasonality and lifestyles of *F. affinis* and *C. peregrina* along the central-eastern coast of Sicily has been reported. These results were interesting since reported information on these two species different from the cited literature: 1) both species along the Ionian coasts of Sicily may be present in any season of the year with a high number of specimens; 2) *F. affinis* along the Ionian

coasts of Sicily is more abundant, and probably more competitive, than *C. peregrina*; 3) the two species showed a less photophilous lifestyle than that usually reported in literature, since in this study both were found in a deeper bathymetric range; 4) if our hypotheses are correct, *F. affinis* and *C. peregrina* are warm-water species and their strong presence in cold seasons might be used as an indicator of the increase in the seawater temperature.

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Saproxylic beetles of conservation interest in the Calabrian side of the Pollino National Park (Calabria, Italia): *Lucanus tetraodon* Thunberg, 1806, *Osmoderma italicum* Sparacio, 2000, *Cerambyx cerdo* Linnaeus, 1758 and *Rosalia alpina* (Linnaeus, 1758) (Coleoptera Lucanidae Cetoniidae Cerambycidae)

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ABSTRACT	In this article an update on the distribution of Coleoptera *Lucanus tetraodon* Thunberg, 1806 (Lucanidae), *Osmoderma italicum* Sparacio, 2000 (Cetoniidae), *Cerambyx cerdo* Linnaeus, 1758 and *Rosalia alpina* (Linnaeus, 1758) (Cerambycidae) in the Calabrian side of the Pollino National Park is described. Data regarding the ecological part and the conservation status will be presented for each species. Therefore, of particular interest it can be noted: the presence in nine locations of *L. tetraodon*, an uncommon species and with little-known distribution; the discreet diffusion of *O. italicum*, rare and with a very restricted area, found in thirteen locations up to over 1800 m of altitude; *C. cerdo* for the Calabrian side of the National Park of Pollino; the first data for the presence of *R. alpina* at 1900 m altitude, the highest recorded in Italy and the discovery of larval stages on Italian alder (*Alnus cordata*), never ascertained in Italy. The Pollino National Park is confirmed as one of the areas of greatest national importance for the conservation of these xylophagous species linked to old forests.

KEY WORDS	Coleoptera; Pollino National Park; Calabria; Italy; saproxylic beetles; distribution; ecology.

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INTRODUCTION

The Pollino National Park, established in 1993, is the largest protected area in Italy, with a coverage of 192.565 ha, divided between Calabria and Basilicata. The Calabrian side has an extension of 103.915 ha and includes three mountain groups, the Ciagola chain that extends toward west in the hydrographic right of the Lao River, between Laino Borgo and Santa Domenica Talao, the Pollino in the central that marks the border between Basilicata and Calabria and the Orsomarso mountains that extend in the southwestern portion between the locality of the same name and Serra La Vriglia at Belvedere Marittimo. The maximum elevation is represented by the Serra Dolcedorme (2267 m), the

highest peak of the Pollino. From a geological point of view, the substratum consists almost entirely of limestone soils belonging to three carbon units, that of Lungro-Verbicaro, that of Pollino-Ciagola and that of Cetraro (Iannace et al., 2005; 2007); near San Donato di Ninea there are also clay shale belonging to the San Donato unit (Ietto & Ietto, 2011).

With regard to vegetation, the forest environments occupy 56% (about 58.000 ha) of the Calabrian side. The most spread typologies are the beech forests, that cover an area from 1300–1400 m up to almost 2000 m; from 600–700 m up to 1300 m, depending on the type of soil and exposure, mixed mesophilous broadleaf woods. The most common species are: manna ash *Fraxinus ornus* L. (1753); European hop-hornbeam, *Ostrya carpinifolia* Scop. (1772); Italian alder, *Alnus cordata* (Loisel.) Duby (1828); field maple, *Acer campestre* L. (1753); Italian maple *Acer opalus* Mill. (1768); sycamore *Acer pseudoplatanus* L. (1753), but also oaks: (especially Turkey oak, *Quercus cerris* L. (1753), downy oak, *Quercus pubescens* Willd. (1796) and holm oak woods *Quercus ilex* L. (1753); instead, the reforestation of conifers and riparian vegetation is not very extensive (Gargano et al., 2012; Valicenti & De Vivo, 2015). These forests, as happened throughout Italy, have been widely exploited, but various old parts has remained, which represent a shelter for saproxilic insects, largely saved from cutting. This is probably due to their position, that was difficult to reach. In the case of oak-woods, they were also maintained for the use of the acorn, both for food and as forage for pigs.

Thanks to a study on the species of insects of community interest present in the Natura 2000 network sites (PO FESR 2014-2020), promoted by the Environment and Territory Department of the Calabria Region and by the Pollino National Park Authority, in the two-year period 2018-19, careful research was carried out on the presence and distribution of *Cerambyx cerdo* Linnaeus, 1758, *Rosalia alpina* (Linnaeus, 1758), *Osmoderma italicum* Sparacio 2000 and *Lucanus tetraodon* Thunberg, 1806 and on the Calabrian side of the Pollino National Park.

The aim of this contribution is to outline an updated picture on the distribution of these species in addition to providing news on ecology, considering that the data available for the southern areas of these species are very poor.

MATERIAL AND METHODS

Samples

The research was carried out between July 2018 and August 2019 in all the Calabrian side of the Pollino National Park (Fig. 1).

Cerambix cerdo has been sampled either through direct adult research and / or remains in suitable habitats near large and old oak trees. This was accomplished either with the use of falling-fall air traps, or by using mature fruit traps (Trizzino et al., 2013; Bologna et al. in Stoch & Genovesi, 2016; Redolfi De Zan et al., 2017).

Rosalia alpina has been investigated through direct research into perishable and dead beech trees, living or with dead portions of them (Trizzino et al., 2013; Bologna et al. in Stoch & Genovesi, 2016).

To estimate the size of populations, in three localities with at least five dead or perishable trees distributed over one area of one hectare (Pollino: Fagosa to Fosso del Vascello and Fagosa Plan; Orsomarso Mountains: Frida and Campicello), three visits were made within two weeks and during these visits, these insect populations were counted with photographic marking and recapture.

Osmoderma italicum has been investigated both through direct adult search on logs and / or cavities and remains, larva and cocoons in woody beads, or by using attractive interception traps (BCWT, black cross window trap) (Trizzino et al., 2013; Bologna et al. in Stoch & Genovesi, 2016). The BWCT, set with the pheromone (γ-decalactone), were placed in a minimum number of six, at least 50 m apart; the traps were checked in alternate days for at least one week.

Lucanus tetraodon has been sampled through direct adult and / or remains on logs and / or cavities; activities have always taken place in the afternoon, when the species is more active (Trizzino et al., 2013; Bologna et al. in Stoch & Genovesi, 2016; Bardiani et al., 2017).

Methods

For each species a label divided into two paragraphs is reported: in the first (Exhibit), the examined materials, the number, the sex of the sampled species, the collection place, the geographical coordinates, the altitude, the municipality, the acronym of the province (in brackets) and the col-
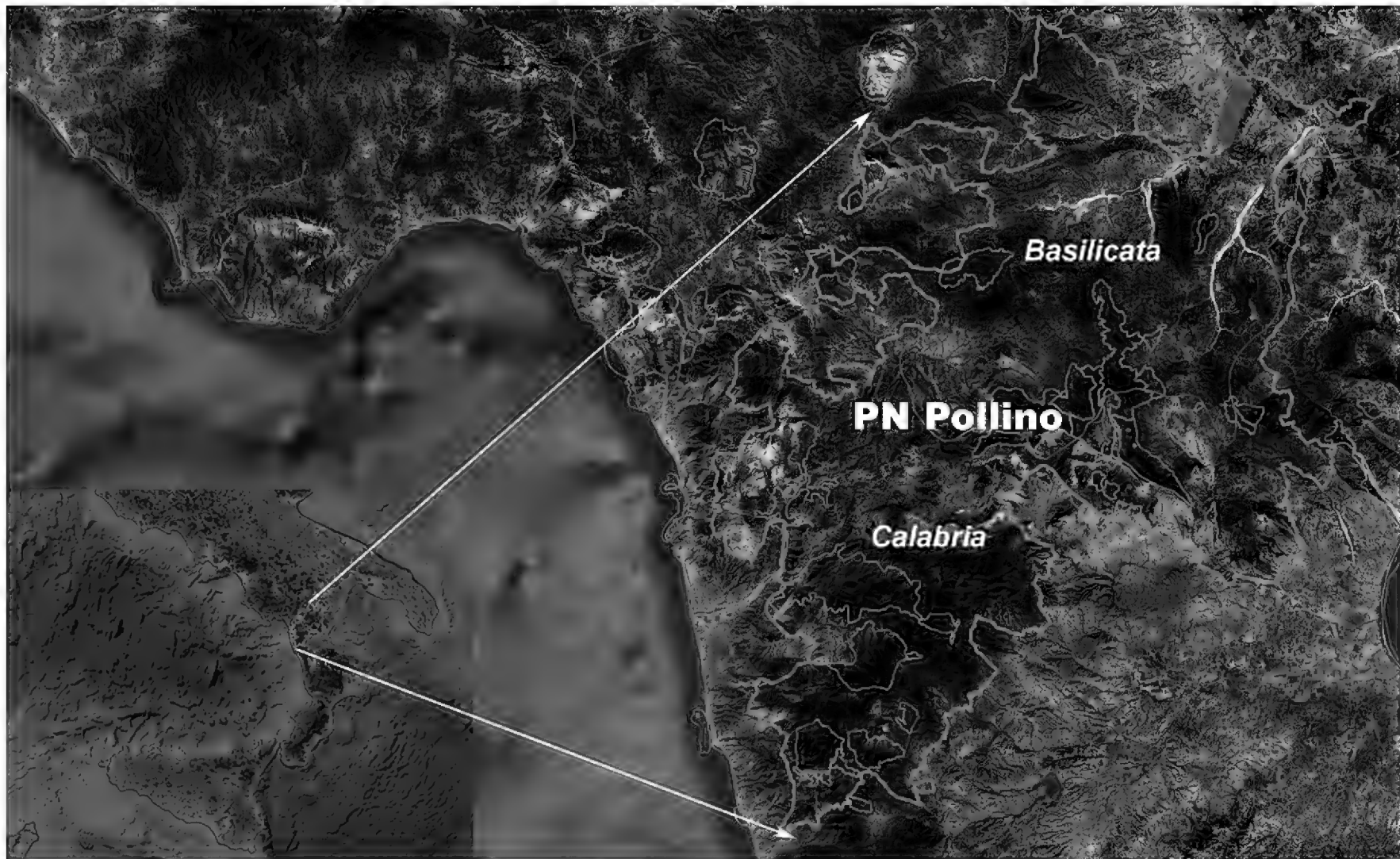
Fig. 1. Area of study. Red line is the limit of the PN Pollino; the blue line is the limit between regions; the orange lines delimit the Sites of Community Importance.

lector/s (SP= vidit/legit S. Piazzini; MT= vidit/legit M. Tamburini; SP & MT= viderunt/legerunt S. Piazzini & M. Tamburini; FR= vidit/legit F. Rotondaro; VM= vidit/legit V. Marchianò; VM & SP= viderunt/legerunt V. Marchianò & S. Piazzini.) are listed; in the second (Observations), information on the distribution, biology, ecology and status of conservation of the examined species are provided and elaborated. Abbreviation ZSC = SAC, Special Area of Conservation.

For reasons related to the conservation of the species, the precise location of the sites has not been indicated, omitting to indicate the latter in the geographical coordinates of the collection locations.

RESULTS AND DISCUSSIONS

LUCANIDAE

Lucanus tetraodon Thunberg, 1806 (Fig. 2)

EXAMINED MATERIAL. 1 ♂ adult, Chain of Ciagola, Contrada Pedali, 39°56'N 15°54'E, 650 m, Laino Borgo (CS), 8.08.19, MT; 1 ♂ adult and 1 ♀ adult, Chain of Ciagola, Gada Mountain, 39°56'N 15°54'E, 970 m, Laino Borgo (CS), 31.07.19, SP; 1 ♂ adult, Pollino, at Fonte Scosa, 39°49'N 16°20'E, 700 m, Francavilla Marittima (CS), 26.07.19, SP; 1 ♂ adult and 1 ♀ adult, Pollino, at S. Maria delle Armi, 39°50'N 16°21'E, 1050 m, Cerchiara di Calabria (CS), 26.07.19 e 10.08.19, SP; 1 ♀ adult, Mountains of Orsomarso, Carpinosa, 39°45'N 15°59'E, 750 m, San Donato di Ninea (CS), 12.08.19, SP; 1 ♂ adult, Mountains of Orsomarso, Casiglia, 39°41'N 16°00'E, 985 m, San Sosti (CS), 25.07.19, SP; 1 ♂ adult and 1 ♀ adult, Mountains of Orsomarso, near Piano di Marco, 39°41'N 16°00'E, 1035 m, San Donato di Ninea (CS), 25.07.19, SP; 2 ♂ adults and 2 ♀ adults, Campicello, 39°39'N 15°58'E, 1132 m, Mottafollone (CS), 28.07.19 e 11.08.19, SP; 1 ♂ adult, Mountains of Orsomarso, Monte Spina Santa, 39°39'N 15°58'E, 1138 m, Sant'Agata di Esaro (CS), 28.07.19, SP.

OBSERVATIONS. Species present in north-eastern Spain, in Provence (France), in Corsica and in a large part of Italy, from the Ticino valley (Piedmont

Figure 2. *Lucanus tetraodon* near Fonte Scosa, Pollino (photo S. Piazzini).

and Lombardy) to the south up to include Sicily, Sardinia and Elba; there are some vague and dated reports for Algeria and the Balkans (Albania) which however need confirmation. *L. tetraodon* is a saproxylophagous beetle, linked to thermophilic and mesophilous broadleaf woods, generally of cork oak *Quercus suber* L. (1753), holm oak and other species of oaks, but also of other Fagaceae, from sea level up to 1300 m of altitude. The larva develops, up to 3-4 years, in dead wood in the stumps of old plants. The period of greatest adult activity is probably between June and August, mainly in the afternoon and evening (Sforzi & Bartolozzi, 2001; Zilioli & Pittino, 2004; Beltrán & Beltrán, 2009; Nieto et al., 2010; Bardiani et al., 2017; Sindaco et al., 2019).

Although it is a sub-endemic Italian species and generally less common than the stag beetle, *Lucanus cervus* Linnaeus, 1758, it is poorly known in terms of distribution in southern Italy (known for only 11 locations in Calabria; Ruffo & Stoch, 2007). Concerning ecology, it is not unfortunately mentioned in any of the main fauna protection reg-

ulations (in contrast to *L. cervus* included in Annex II of Directive 92/43 / EEC and in Annex III of the Bern Convention) despite the undeniable **interest** from the zoogeographic point of view.

L. tetraodon, known so far for the Calabrian side of the Pollino Park only for three dated detections (Valico di Campotenese, Convent of Colloreto and Monte Pollino) (Ruffo & Stoch, 2007), was found in nine unpublished locations, three of which fall in as many ZSC (Carpinosa: IT9310028 Valley of the Abatemarco River; Piano di Marco in the homonymous ZSC IT9310022; Campicello: IT9310027 Rosa River). The sites are between 600 m and 1140 m and are all characterized by the presence of woods with a prevalence of Fagacee (holm oak, downy oak, turkey oak, beech) with the presence of old plants, in some cases even old, generally large and with stumps or other portions dead or perishing.

The host plants of the larva are represented in three cases by the turkey oak, in two cases by the downy oak, in one case by the holm oak and in three cases by the beech; at lower altitudes the species

also lives inside the wood, while going up in altitude it seems to prefer much more open situations, with the host plants placed in full sun.

Of particular interest are the cases of Campicello and Monte Spina Santa, where the host plant is represented by beech instead of oak species; in these two locations *L. tetraodon* lives within a short distance of *R. alpina* but the two species compete on a trophic level; in fact in the microhabitat (i.e., the dead plant) the two species are mutually exclusive and *L. tetraodon* is found in the broadest and sunniest cuttings. *Rosalia alpina*, instead, is found on dead plants in smaller, less sunny cuttings or at the edge of the forest. In three locations (Piano di Marco, Spina Santa Mountain, Gada Mountain), this species lives in the same microhabitat with *O. italicum* and in two locations (at Fonte Scosa, near S.Maria delle Armi) with *O. italicum*, *M. asper*, *C. cerdo* and *Cerambyx welensii* (Küster, 1846); in these cases the presence of one xylophagous species does not exclude the other, probably because each one uses a different part of the dead plant (*L. tetraodon* the stump, *O. italicum* the mould in the cavities, *M. asper* the dead branches fallen on the ground and the *Cerambyx* the dead parts still standing).

In the area under investigation, the main threats are removal of dead trees and fires.

CETONIIDAE

Osmoderma italicum **Sparacio, 2000** (Fig. 3)

EXAMINED MATERIAL. 1 ♀ adult, Chain of Ciagola, Gada Mountain, 39°56'N 15°54'E, 1030 m, Laino Borgo (CS), 31.07.19, SP; 1 ♂ adult, Pollino, Sparviere Mountain, 39°55'N 16°21'E., 1300 m, Alessandria del Carretto (CS), 14-16.08.18, SP; 1 ♀ adult, Pollino, Piano di Acquafredda, 39°54'N 16°13E, 1860 m, Cerchiara di Calabria (CS), 8.08.18, VM; 1 ♀ adult, Pollino, near Colle Marcione, 39°52'N 16°17'E, 1261 m, Frascineto (CS), 21.07.19, FR; 1 ♀ adult, Pollino, near Fonte Scosa, 39°49'N 16°20'E, 700 m, Francavilla Marittima (CS), 22.07.19, SP & MT; 1 ♀ adult, Pollino, Cernostasi, 39°50'N 16°19'E, 785 m, Francavilla Marittima (CS), 18.07.19, SP & MT; 1 ♀ adult, Pollino, near S. Maria delle Armi, 39°50'N 16°21'E, 1050 m, Cerchiara di Calabria (CS), 26.07.19 e 10.08.19, SP; complete remains of 1 ♀ adult, Mountains of Orsomarso, side N di Serra della Lupara, 39°48'N 16°04'E, 1680 m, Saracena

Figure 3. *Osmoderma italicum* near Timpone Vaccaro, Mountains of Orsomarso (photo S. Piazzini).
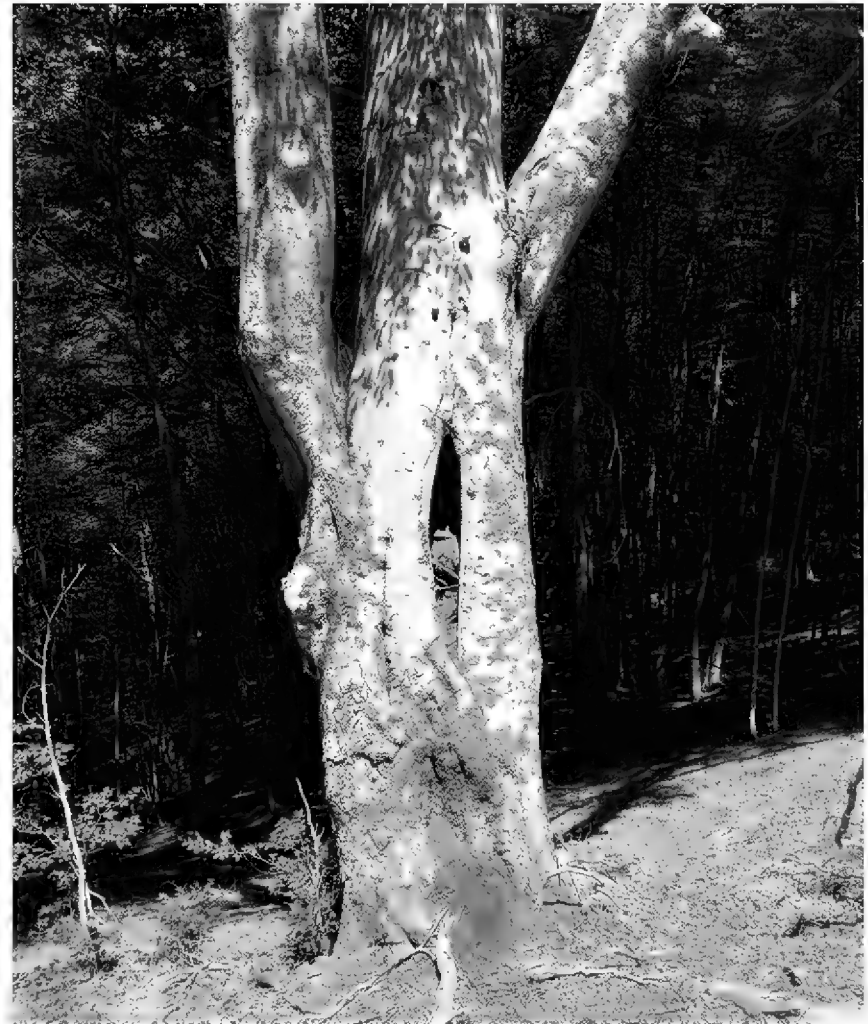
Figure 4. Habitat of *O. italicum* near M. Petricelle (photo S. Piazzini).

(CS), 14.08.19, SP; 1 ♂ adult, Mountains of Orso-marso, side E di Timpone Vaccaro, 39°49'N 16°02'E, 1255 m, Saracena (CS), 22.08.18, SP; 1 ♀ adult, Mountains of Orsomarso, San Donato di Ninea, 39°43N 16°02'E, 1091 m, San Donato di Ninea (CS), SP & MT; 1 ♀ adult, Mountains of Or-somarso, at Piano di Marco, 39°41'N 16°00' E, 1006 m, San Donato di Ninea (CS), 25.07.19, SP; complete remains di 1 ♂ adult, Mountains of Orso-marso, Spina Santa Mountain, 39°39'N 15°58'E, 1138 m, Sant'Agata di Esaro (CS), 28.07.19, SP; complete remains di 1 ♀ adult, Mountains of Orso-marso, side N di Monte Petricelle, 39°39'N 15°54'E, 1462 m, Buonvicino (CS), 7.08.19, SP & MT.

Observations. Endemic species of southern Italy, currently known for few places in Campania, Puglia, Basilicata and Calabria. Some authors, fol-lowing molecular investigations, have confirmed the affinity of *O. italicum* with *O. eremita* (the latter spread in western Europe) and consider *O. italicum* a taxon on the border between an allopatric species and a semi-species in the process of being differen-tiated (Audisio et al., 2007; 2009). *O. italicum* is a saproxylophagous beetle, tied to broad-leaf woods with the presence of large rotting trees characterized by medium and large cavities with degraded necro mass inside in the form of woody mould (which feeds the larva), where it lays its eggs. From the available data, *O. italicum*, which is found mainly from high hills (600 m) up to 1600 m, seems more tied to woods and forests than the congeneric *O. eremita* who is found, however, very often, in agro-ecosystems with the presence of rows or sporadic willow plants. The period of greatest activity of adults is probably between June and August (Trizzino et al., 2013; Mazzei et al., 2014; Bologna et al. in Stoch & Genovesi, 2016; Mazzei & Brand-mayr, 2017). The species is mentioned in Annexes II and IV of Directive 92/43 / EEC and is consid-ered "In danger" in Italy and "Near Threatened" globally (Nieto et al., 2010 in IUCN, 2020; Ron-dinini et al., 2013 in IUCN Italian Committee, 2020).

Osmoderma italicum, known so far in fourteen locations across the entire range of which only three falling on the Calabrian side of the Pollino Park, namely Morano Calabro, Alessandria del Carretto and La Mula Mountain (Ruffo & Stoch, 2007; Mazzei et al., 2014; Mazzei & Brandmayr, 2017),

was found in thirteen locations, eleven of which new: six locations fall within the ZSC (IT9310019 Monte Sparviere; IT9310014 Fagosa-Timpa del-l'Orso; IT9310022 Piano di Marco; IT9310027 Rosa River; IT9310032 Serrapodolo). The detected places, located between 700 m and 1800 m, are all characterized by the presence of deciduous forest (beech, downy oak, turkey oak, chestnut, Italian alder) with the presence of large, old trees, with large portions and cavities in the trunk (also located at the base) or in the branches, containing mould of partially decomposed wood (Fig. 4), confirming the greater forestry attitudes of *O. italicum* compared to *O. eremita*. The host plants of the larva are rep-resented in seven cases by beech, in three cases by downy oak and in one case each by turkey oak, chestnut and Italian alder.

From the available data it seems that the flicker occurs from the second half of July, in the places with lower altitudes, and until the middle of August, in the higher places.

O. italicum lives with other xylophagous in-sects: in five cases with *R. alpina*, in two cases with *L. tetraodon* and in one case with *C. cerdo* and *M. asper*.

In relation to the territory under investigation, the main pressures / threats are represented by the cutting of dead trees, by the excessive human dis-turbance for outdoor activities and by the collection of insects.

CERAMBICYDAE

Cerambyx cerdo Linnaeus, 1758

Examined material. Complete remains of 2 adults ♂ and 1 adult ♀, Pollino, near S. Maria delle Armi, 39°50'N 16°21'E, 1050 m, Cerchiara di Cal-abria (CS), 26.07.19 e 10.08.19, SP & MT; Com-plete remains 1 ♂ adult, Mountains of Orsomarso, near Piano di Marco, 39°41'N 16°00' E, 1035 m, San Donato di Ninea (CS), 25.07.19, SP.

Observations. Species widespread throughout central and southern Europe, from Spain to the east to Ukraine, in northern Africa, the Caucasus and Minor Asia. In Italy it is detected in all regions ex-cept Val d'Aosta. *C. cerdo* is linked to forests with a prevalence of oaks with the presence of large trees that are still viable but with perishing parts; can also be found more rarely on chestnut, willow, elm, wal-

nut and ash. It lives from sea level up to 2000 m of altitude on the Atlas but in Italy it seems not to exceed 1300 m. The larva develops in large oak trunks (at least 60 cm in diameter) by digging long tunnels and feeding on wood; reaches maturity at three to five years, when it metamorphoses. The period of major activity in the adults is between May and August (Buse et al., 2016 in IUCN, 2019; Stoch & Genovesi, 2016; Redolfi De Zan et al., 2017). The species is listed in the attachments II and IV of Directive 92/43/CEE and it is considered as "Least Concern" at Italian and global levels (Rondinini et al., 2013 in IUCN Comitato Italiano, 2020; Buse et al., 2016 in IUCN, 2020).

Cerambix cerdo, for the time being, is not being alerted in the Calabrian side of Pollino Park, as it has been detected in two places, one in the ZSC IT9310022 Piano di Marco. The detection places are both located just over than 1000 m above sea level and characterized by the presence of forest with a prevalence of oaks (downy oak at S. Maria delle Armi and Turkey oak at Piano di Marco) with the presence of old, large plants, still viable but with some perishing parts. In both places *C. cerdo* lives with *Morimus asper* (Sulzer, 1776), *O. italicum* e *L. tetraodon*.

Extensive research should include sampling of the repeated species for several years, the low presence of this longhorn beetle in the area of survey is likely to be attributed to both the relatively limited extent of oak forests and to the low presence of large-size old oak trees, the primary habitat of the species.

In relation to the investigated territory, the main pressure is currently being represented by possible fires, given that the underlying hilly areas are often affected by the fire, and with regard to S. Maria delle Armi, the last one happened in July 2019, arriving almost in lapping such a place. Other threats appear to be the removal of dead and perishing trees and large ones derived by intensified agriculture, but also from the collection of specimens for collection purposes.

Rosalia alpina (Linnaeus, 1758) (Fig. 5)

EXAMINED MATERIAL. 1 ♂ adult and 1 ♀ adult, Chain of Ciagola, Gada Mountain, 39°56'N 15°54' E, 1030 m, Laino Borgo (CS), 31.07.19, SP; 1 ♂ adult and 1 ♀ adult, Pollino, Sparviere Mountain,

39°54N 16°21'E, 1287 m, Cerchiara di Calabria (CS), 5.08.19, MT; 2 ♂ adult and 2 ♀ adults, Pollino, La Fagosa near Fonte del Vascello, 39°53'N 16°14' E, 1410 m, Castrovillari (CS), 27.07.18, 8.08.18 e 13.08.18, SP & MT; 2 ♂ adults and 4 ♀ adults, Pollino, Piano della Fagosa, 39°55N 16°13' E, 1513 m, Cerchiara di Calabria (CS), 8.08.18, 24.08.18 e 6.09.18, MV & SP; 1 ♂ adult, Pollino, La Montagnola, 39°55N 16°13' E, 1650 m, Cerchiara di Calabria (CS), 6.09.18, MV & SP; 2 ♂ adults and 2 ♀ adults, Pollino, Piano di Badia, 39°53'N 16°14' E, 1325 m, Castrovillari (CS), 27.07.19, SP; 2 ♂ adult and 1 ♀ adults, Pollino, near Colle Sparto, 39°53'N 16°15' E, 1200 m, San Lorenzo Bellizzi (CS), 27.07.19, SP; 1 ♀ adult, Pollino, Piano di Ratto, 39°52'N 16°16' E, 1400 m, Frascineto (CS), 21.07.19 FR, 10.08.19 SP & MT; 1 ♀ adult, Pollino, Colle Ratto, 39°52'N 16°16' E, 1384 m, Frascineto (CS), 10.08.19, SP & MT; 1 ♂ adult and 1 ♀ adult, Pollino, northern side of Timpa del Principe, 39°52'N 16°15'E, 1457 m, Frascineto (CS), 10.08.19, SP & MT; 1 ♂ adult and 1 ♀ adults, Pollino, Pietra Caduta, 39°52'N 16°15'E, 1521 m, Frascineto (CS), 10.08.19, SP & MT; 1 ♂ adult, Pollino, Costa di Malaverna, 39°52'N 16°15'E, 1754 m, Frascineto (CS), 10.08.19, SP & MT; 1 ♂ adult, Pollino, southern side of Serra Dolcedorme, 39°53'N 16°13'E, 1850 m, Castrovillari (CS), 13.08.19, SP; 1 ♀ adult, Pollino, southern side of Serra del Prete, 39°54'N 16°09' E, 1540 m, Morano Calabro (CS), 26.08.18, VM & SP; 1 ♂ adult and 1 ♀ adult, Pollino, eastern side of Serra del Prete, 39°54'N 16°10' E, 1790 m, Morano Calabro (CS), 26.08.18, SP; 1 ♂ adult, Mountains of Orsomarso, northern side of Monte Palanuda, 39°49'N 16°00'E, 1535 m, Mormanno (CS), 25.08.18, SP; 1 ♀ adult, Mountains of Orsomarso, northern side of Serra della Lupara, 39°48'N 16°04'E, 1680 m, Saracena (CS), 14.08.19, SP; 1 ♂ adult & 2 ♀ adults, Mountains of Orsomarso, southern side of Monte Caramolo, 39°47' N 16° 05'E, 1690 m, Saracena (CS), 14.08.19, SP; 1 ♂ adult and 2 ♀ adults, Mountain of Orsomarso, eastern side of Timpone Vaccaro, 39°49'N 16°02'E, 1255 m, Saracena (CS), 22.08.18, 6.08.19 e 15.08.19, SP; 1 ♂ adult, Mountains of Orsomarso, at Rifugio Mare Piccolo, 39°48'N 16°01'E, 1185 m, Orsomarso (CS), 6.08.19, SP; 1 ♀ adult, Mountains of Orsomarso, Serra Paratizzi, 39°43'N 15°59'E, 1763 m, San Donato di Ninea (CS), 7.09.18, SP; 1 ♂ adult and 2 ♀ adults, Moun-

tain of Orsomarso, Frida, 39°42'N 16°00'E, 1300 m, San Donato di Ninea (CS), 9.08.18, 14.08.18 e 23.08.18, SP & MT; 1 ♀ adult, Mountain of Orsomarso, Valley of Vespa, 39°44'N 16°01'E, 1695 m, San Donato di Ninea (CS), 7.09.18, SP; 1 ♂ adult and 1 ♀ adult, Mountains of Orsomarso, Cozzo del Pellegrino, 1900 m, San Donato di Ninea (CS), 7.09.18, SP; 1 ♀ adult, Mountains of Orsomarso, at Carpinosa, 39°45'N 15°59'E, 800 m, San Donato di Ninea, 12.08.19, SP; 1 ♀ adult, Mountains of Orsomarso, Nona, 39°45'N 15°59'E, 1020 m, San Donato di Ninea (CS), 12.08.19, SP; 1 ♀ adult, Mountains of Orsomarso, Sferracavallo, 39°41'N 15°59'E, 1285 m, San Donato di Ninea (CS), 25.07.19, SP; 1 ♀ adult, Mountains of Orsomarso, Piano di Marco, 39°41'N 16°00'E, 1050 m, San Donato di Ninea (CS), 6.08.19, MT; 1 ♂ adult and 2 ♀ adults, Mountains of Orsomarso, Strette di Prezzamano, 39°39'N 15°59'E, 1160 m, Mottafollone (CS), 7.08.18, 18.07.19 e 28.07.19, SP & MT; 1 ♀

adult, Mountains of Orsomarso, Campicello, 1080 m, 39°39'N 15°58'E, 1080 m, Mottafollone (CS), 28.07.19, SP; 1 ♂ adult, Mountains of Orsomarso, Monte Prezzamano, 39°39'N 15°59'E, 1195 m, Mottafollone (CS), 28.07.19, SP; 1 ♂ adult, Mountains of Orsomarso, Pietra Portusata, 39°39'N 15°58'E, 1167 m, Mottafollone (CS), 28.07.19, SP; 1 ♀ adult, Mountains of Orsomarso, Scrigna dell'Asino, 39°39'N 15°57'E, 1178 m, Sant'Agata di Esaro (CS), 28.07.19, SP; 1 ♀ adult, Mountains of Orsomarso, La Montea, 39°39'N 15°57'E, 1650 m, Mottafollone (CS), 11.08.19, SP; 1 ♀ adult, Mountains of Orsomarso, Pietra del Cisso, 39°40'N 15°54'E, 1372 m, Buonvicino (CS), 7.08.19, SP & MT; 1 ♀ adult, Mountains of Orsomarso, northern side of Monte Frattina, 39°40'N 15°54'E, 1475 m, Buonvicino (CS), 7.08.19, SP & MT; 1 ♂ adult and 1 ♀ adult, Mountains of Orsomarso, northern side of Monte Petricelle, 39°39'N 15°54'E, 1462 m, Buonvicino (CS), 7.08.19, SP & MT; 1 ♀ adult,

Figure 5. *Rosalia alpina* near Piano della Fagosa, Pollino (photo S. Piazzini).

Mountains of Orsomarso, northern side of Monte Petricelle, 39°39'N 15°55'E, 1750 m, Buonvicino (CS), SP & MT.

OBSERVATIONS. Species widespread in central and southern Europe, from southern Scandinavia to the Iberian peninsula and Italy, from Russia through the Balkan peninsula to northern Turkey; in the southern portion of the range it is present with a very fragmented distribution. In Italy it is alerted in all regions with the exception of Sardinia and the Aosta Valley, but is more frequent along the Apennine arc. *Rosalia alpina* is mainly linked to mature beech woods, generally from high hills (500 m) up to mountain altitudes (1500 m).

It prefers old woods with a prevalence of beech, *Fagus sylvatica*, with the presence of perishing and dead plants, and the larva feeds on those routings; more rarely it lives also on other broad-leaf trees (in particular *Acer*, *Quercus*, *Ulmus, Fraxinus*, *Castanea* and *Alnus*). The period of flickering varies according to altitude and climate, but generally between May and August (Mazzei et al., 2013; Trizzino et al., 2013; Campanaro et al., 2017; Mazzei & Brandmayr, 2017). The species is mentioned in Annexes II and IV of Directive 92/43 / EEC and is considered "Almost threatened" in Italy and "Least Concern" globally (Verdugo et al., 2016 in IUCN, 2020; Rondinini et al., 2013 in IUCN Italian Committee, 2020).

In the Calabrian side of the Pollino National Park, *R. alpina* was known with certainty in only two ZSC, IT9310014 La Fagosa-Timpa dell'Orso and IT9310021 Cozzo del Pellegrino (Mazzei et al., 2013; Mazzei & Brandmayr, 2017). On the basis of our findings, however, it was rather widespread, even common in some more limited areas and having been found in thirty-eight locations, almost all unpublished. It is mainly located in La Fagosa and Orsomarso, especially on the Ionian side, while it is probably more isolated at Sparviere Mountain, at

Figure 6. Habitat of *Rosalia alpina* near Serra del Prete, Pollino (photo S. Piazzini).

the northeastern end of Pollino and at Gada Mountain, in Chain of Ciagola.

Despite the good diffusion, the sampling carried out in four locations (Fosso del Vascello, Piano della Fagosa, Acqua di Frida and Campicello) with CMR, however, revealed modest populations (maximum eight specimens). These results are quite similar to those obtained with the same methods in the Casentinian Forests National Park, but very different from those observed in the Abruzzo National Park, where up to 172 specimens were detected on transepts with 15 dead plants (Campanaro et al, 2017). This significant difference, however, could not depend on the actual size of the populations but on their distribution in the habitat: it is possible that *R. alpina* is widespread but with smaller metapopulations where there is a larger habitat (NP of Pollino and NP of Casentinian Forests), while it is present with more abundant point populations (NP of Abruzzo) where the habitat is more localized.

Twenty-seven locations fall into Special Conservation Zones: eleven in the ZSC IT9310014 Fagosa-Timpa dell'Orso IT9310014, five in the ZSC IT9310032 Serrapodolo, four in the ZSC IT9310028 Valle del Fiume Abatemarco, three in the ZSC IT9310022 Piano di Marco, one in the ZSC IT9310023 Valle of the Argentino River, one in the ZSC IT9310027 Rosa River and one in the ZSC IT9310029 La Montea. The detected places are located from 800 m up to 1900 m, which represents the highest altitude ever recorded in Italy, and are all characterized by mature or old woods, generally with a prevalence of beech, with the presence of at least three to four dead plants of large size (standing or on the ground) or perishing distributed in a minimum area of 0.5 ha.

Rosalia alpina was detected in thirty-five cases out of thirty-eight of beech (92%). Numerous females have been observed in egg-laying who seem to prefer the stubs of large plants, dead on their feet and broken for a relatively short time (Fig. 6). In three cases (Frida, Piano di Marco and Sparviere Mountain) it was detected on Italian alder (8%) and in two of these it was observed to lay eggs, a fact that represents the first case documented in Italy confirming a relative polyphagia of the species, which probably occurs in portions of old woods and in marginal areas not too far from the primary habitat represented, in fact, by beech woods. From the

available data, the flickering occurs from the beginning of July, at the lowest altitudes (around 1000 m) until the end of August at the highest altitudes (1800–1900 m). *R. alpina* was found in syntopia with *O. italicum* in six locations, while in two of these (Piano di Marco and Monte Gada) it also lives with *L. tetraodon and M. asper.*

In relation to the territory under investigation, the greatest threats seem to be represented by the illegal cutting of perishing plants, the removal of dead trunks and fires; however, illegal collection for collection purposes should not be underestimated.

CONCLUSIONS

From the above results it is clear that the Pollino National Park plays a primary importance role at national level for the protection of *L. tetraodon*, *O. italicum*, *C. cerdo* and *R. alpina*. It is an entity of conservation interest pursuant to Directive 92/43/ EEC as priority (*R. alpina*), and endemic, sub-endemic or of biogeographical interest. In all cases, these are umbrella species whose protection makes it possible to conserve the other saproxylic species and with them the old forests, habitats that are gradually decreasing on a global level, vital for the survival of a large number of animal species among the invertebrates and vertebrates. This is possible, however, only through the promotion of specific studies and research that highlight the areas of greatest naturalistic value and allow the authorities in charge to take the necessary actions for rigorous protection without which the future of these habitats and species is seriously at risk.

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Duration of pseudo-stalked barnacles (*Xenobalanus globicipitis*) on a New Zealand Pelagic ecotype orca (*Orcinus orca*), with comments on cookie cutter shark bite marks (*Isistius* sp.); can they be used as biological tags?

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ABSTRACT   This is the first published report of a New Zealand Pelagic ecotype orca (*Orcinus orca* Linnaeus, 1758, killer whale, Mammalia Cetacea) as a host for the pseudo-stalked barnacle (*Xenobalanus globicipitis* Steenstrup, 1852, Crustacea Coronulidae). The barnacles were documented on an adult female and she hosted >79, >3.5 times higher than any other orca worldwide. They were distributed on her dorsal fin (*n*=3), pectoral fins (*n*=>36) and tail flukes (*n*=>40), with a higher density on her right appendages (*n*=>48) compared to her left (*n*=>28). We also document, for the first time, the longevity of *X. globicipitis* hosted on an orca, with a minimum duration of 36 days. We provide a global overview of the distribution of *X. globicipitis* on orca, based on historic and recent publications. In previous reviews (spanning 111 years of records) *X. globicipitis* were documented in ten regions, while we add ten more regions, in just 13 years. This leads us to speculate as to the causes of this rapid increase, which may be linked to observer bias, improved research tools, a change in the distribution of either *O. orca* or *X. globicipitis*, with distribution of the latter perhaps influenced by oceanic conditions such as marine heatwaves and acidification triggered by climate change. As such, we discuss if either pseudo-stalked barnacles (or bite marks from cookie cutter sharks, *Isistius* sp., Chondrichthyes Squaliformes) can be used as biological tags or markers for orca ecotypes. We recommend separate management plans for orca ecotypes.

KEY WORDS   Cookie cutter shark *Isistius* sp.; killer whale *Orcinus orca*; pseudo-stalked barnacle; *Xenobalanus globicipitis*; Pelagic ecotype; spatial and temporal.

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IINTRODUCTION

Orca (*Orcinus orca* Linnaeus, 1758, also known as the killer whale, Mammalia Cetacea) are distributed globally, with different ecotypes recognised in many locations, including New Zealand (NZ). For definitions of ecotypes and some examples see Ford et al. (2014) and Riesch (2016). The NZ Pelagic ecotype is recognised by key features such as foraging on marine mammals, pigmentation variations and a high prevalence of cookie cutter shark *Isistius* sp. bite marks (Visser & Cooper, 2020a, 2020b).

The pseudo-stalked barnacle (*Xenobalanus glo-bicipitis* Steenstrup, 1852, Crustacea Coronulidae), hereafter referred to as *Xenobalanus* Steenstrup, 1852 has been proposed as a biological tag/marker for cetaceans (Karuppiah et al., 2004; Aznar et al., 2005; Hartny-Mills, 2015; Siciliano et al., 2020), including orca (Whitehead et al., 2014; Matthews et al., 2020). *Xenobalanus* are filter-feeding cirripedes which are obligate-cetacean epizootics (Fertl & Newman, 2018) and the barnacle has been documented on at least 34 species of free-ranging and stranded cetaceans (Kane et al., 2008). On orca, they have been documented in a range of geographic locations (Fig. 1). In the following list we use these abbreviations: IS = *in situ*, where specimens have been documented (e.g., photographed) in the field (on live or stranded hosts) and identification to species level is confirmed or presumed based on external morphological characteristics and attachment to a cetacean host; ES = *ex situ*, where specimens have been collected from a cetacean host and identification to species level is confirmed from morphological external/internal characteristics and/or dissection and/or DNA:

Australia (Donnelly et al., 2018, IS), Brazil (Siciliano et al., 2020, IS), California (Samaras, 1989, ES; Black et al., 1997, IS), Canadian Arctic (Matthews et al., 2020, IS), Canary Islands (Foote et al., 2011, IS), Chile (Cortés Peña, 2019, IS), Costa Rica (Fertl et al., 1996, IS), Eastern Tropical Pacific (Pitman et al., 2007, IS; Kane et al., 2008, IS; Olson & Gerrodette, 2008, IS), Galápagos Islands (Denkinger & Alarcon, 2017, IS), Gibraltar (CIRCE, 2020, IS) we note that an earlier record attributed to Gibraltar should be listed as Spanish, see S-1 for details, Guatemala Basin (Olson & Gerrodette, 2008, IS), Japan (Sakai Y. et al., 2009, ES) [we note that Sakai Y. et al. (2009) at page 82, erroneously claim that their record from an orca captured off Kii Peninsula, Japan in "winter, 1988", "*appears* [to be] *the first record of X. globicipitis from killer whale*"; however, the Japan specimen was preceded by the Monaco specimen (Richard & Neuville, 1897) and the Spanish specimen (Gruvel, 1920) (see S-1 for details) and by the California specimen (Samaras, 1989)], Monaco (Richard & Neuville, 1897, ES; Gruvel, 1920, ES; Richard, 1936, ES; Richard & Neuville, 1936, ES); Mexico (Black et al., 1997, IS; Guerrero-Ruiz & Urbán, 2000, IS; Olson & Gerrodette, 2008, IS; Vargas-Bravo et al., 2020, IS), Peru (Olson & Gerrodette, 2008, IS; Pacheco et al., 2019, IS), South Africa

Figure 1. Regions where *Xenobalanus globicipitis* have been documented on orca (1896-2020, see text for details). The 10 regions identified in Rajaguru & Shantha (1992) and Kane et al. (2008) during a 111-year period are indicated by unlabelled grey areas with a dotted border. Since Kane et al. (2008), 10 additional regions (labelled with region name, indicated by grey and striped areas) have been added in just 13 years. The size of the areas approximates the general region only (e.g., the Monaco area is for one record, yet extends outwards).

(Best, 2007, ES; Whitehead et al., 2014, IS) and Spain (Gruvel, 1920, ES; Richard, 1936, ES).

One of the earliest records of *Xenobalanus* on orca is from 27 May 1896, off Monaco in the Mediterranean Sea, when two female orca (5.90 m and 4.10 m in length) from a group of three individuals, were harpooned and killed (Richard & Neuville, 1897). See S-1 for details of this record as Richard (1936) is often erroneously cited as the author.

The authors reported that '*several Xenobalanus were attached to the tail and pectoral of the large Orca*' [translated]. That orca also had large pieces of cetacean(s), including fragments of flesh still covered with skin as well as large pieces of skin, some nearly a metre in length, inside the stomach.

Six years later, on 22 July 1902 off La Chullera, Spain, another female orca (4.70 m long) was harpooned and killed (Gruvel, 1920; Richard & Neuville, 1936) (see S-1 for details of this record as Richard (1936) is often erroneously cited as the author, however both Gruvel (1920) and Richard & Neuville (1936) have details of this capture) and she had an unspecified number of *Xenobalanus* on both pectoral fins as well as on her tail flukes. Inside her stomach were large (1–2 kg) pieces of fish and bones that were speculated to be from tuna or swordfish.

The different food types found in the stomach of these two individuals is indicative of different orca ecotypes (i.e., mammal- and fish-eating populations). As another distinguishing feature, the presence of *Xenobalanus* on orca may be markers for the distribution of and/or different ecotypes (Whitehead et al., 2014; Matthews et al., 2020).

METHODS

Study methods

As part of the long-term (nearly three decades) study of NZ orca, carried out by the Orca Research Trust (www.orcaresearch.org), opportunistic sightings of orca are attended. The date (in yyyymmdd format), location of the encounter, water temperature and environmental conditions are documented, the animals are photographed and/or videoed and behavioural observations collected. Video is typically collected using a HD GoPro Hero® camera on a pole, which can be placed underwater. Subsequently, frames from the video can be extracted

for analysis. In addition to these dedicated research encounters, sighting records (including photographs/videos) are collected on an *ad hoc* basis from citizen scientists and marine mammal tour operators (see Visser, 2000 for details).

Individual orca are identified using congenital and acquired marks/pigmentation and are then assigned catalogue numbers (see Visser, 2000 for details). They are also classified into one of the orca populations documented in NZ waters, based on a range of features such as pigment variations and a prevalence of cookie cutter shark (*Isistius* sp.) bite marks (Dwyer & Visser, 2011; Visser & Cooper, 2020a, 2020b).

Identification of the orca NZOP–005

The subject of this publication is an adult female Pelagic ecotype (catalogue identification number NZOP–005, also known as "Māia", a Māori name meaning "brave", "bold", "capable" and "confident"). She was identified using *inter alia;* (i) asymmetrical saddle patches (Figs. 2, 3) similar to some of the orca described in Mäkeläinen et al. (2013). On the left side she had what is classified as a "smooth" saddle patch (Fig. 2) and on her right side an "open" saddle patch with a "vertical notch" (Fig. 3) as described in Sugarman (1984) and Baird & Stacey (1988). She also had a band of four parallel marks (Figs. 4–6) which, based on their spacing, most likely were a result of "rake marks" from conspecifics teeth; see Scheffer (1969) and Visser (1998) for examples.

Additionally, NZOP–005 had cookie cutter shark bite marks, one of which resulted in an indent which spanned across her spinal ridge, resulting in the scar being visible on both sides of the animal (Figs. 2–6). The bite mark would be classified by Dwyer & Visser (2011) as "*SCAR: completely healed wound, typically with change in original skin pigmentation colour*". See Dwyer & Visser (2011) for details regarding identification of scars and wounds from these sharks. The indent was ~7cm long (based on measurements of similar cookie cutter shark bite marks on necropsied orca, Visser & Orca Research Trust, unpublished data).

Xenobalanus globicipitis

We identified *Xenobalanus* from the morpho-

Figure 2. NZOP–005 on 20150823, photographed off Cape Brett, Bay of Islands, east coast Northland. She was host to three *Xenobalanus globicipitis* barnacles on the trailing edge of her dorsal fin, near the tip (see insert for details of dark pigmentation of the barnacle, one of the distinguishing features of this species). Note the indent in the spinal ridge near the base of the fin which extends into the saddle patch (black arrow). This was from a cookie cutter shark (*Isistius* sp.) bite mark and used to cross match this orca between years and confirm matches from left and right sides. Photo by HG.

logical features visible in photographs and compared those to published images, anatomical drawings and descriptions in published literature (e.g., Steenstrup, 1852; Darwin, 1854; Bane & Zullo, 1980; Rajaguru & Shantha, 1992; Pugliese et al., 2012; Fertl & Newman, 2018; Dreyer et al., 2020). For example, Darwin (1854: 440), states *"The whole surface is smooth, and is formed by rather thin membrane, of an orange colour; but from the colour of the underlying corium, the whole appears of a dark chocolate red, the reflexed hood being rather lighter coloured"*. Bane & Zullo (1980) describe the colour as *"uniform reddish purple color, except for the cream-to-buff-colored basal attachment disk"*. Pugliese et al. (2012) focused on describing the attachment plates and mentioned *"calcified attachment plates"* which display *"foliate projections"*. Darwin's (1854, fig. 4a) is remarkable in its detail and an accurate depiction of what is seen in the dissecting microscope photographs in Pugliese et al. (2012, their Fig. 1), however they describe the shape as *"Each footplate radiates out from the central pedicle and together leading to the formation of what resem-* *bles a flower-shaped rather than star-shaped (Rajaguru and Shanta, 1992) attachment organ"*. Carrillo et al. (2015) provide a close-up photograph of at least eight attachment shells from the flukes of a dead striped dolphin (*Stenella coeruleoalba*, Meyen, 1833) which show star-shaped attachment shells with rounded lobes, similar to a flower shape. Siciliano et al. (2020) also used photographs to identify *Xenobalanus* on cetaceans *in situ*, including orca, and noted *"The genus* Xenobalanus *is monotypic, and the most similar barnacle genus is* Conchoderma *von Olfers, 1814, with four recognized species, of which two,* C. auritum *(Linnaeus, 1767) and* C. virgatum *Spengler, 1789, were registered on cetaceans, settling in hard substrata such as teeth and sessile barnacles. Thus,* X. globicipitis *was identified by its narrower peduncle and darker colouration and its settlement in soft substrate"* [citations excluded].

Therefore, the features we used to assess the specimens *in situ* were *inter alia*; location on the cetacean host (i.e., typically on (or very near to) the trailing edges of orca appendages); smooth skin; cylindrical shaped and flexible body ('stalk'); a red-

brown-purple colouring of the body; a paler coloured 'hood'; cirri; the shell or basal attachment plates being small, radiating out laterally from the base of the body and exhibiting a star/flower-shaped structure with irregular and/or rounded lobes; shell, whitish-cream in colour.

We note that although copepods of the genus *Pennella* may superficially resemble *Xenobalanus*, we believe that the specimens recorded on the orca were *Xenobalanus* based on comparative features (e.g., Fraija-Fernández et al., 2018).

We counted the number of *Xenobalanus* on each appendage by using photographs which were post-processed using machine-learning TopazLabs (https://topazlabs.com/) AI software for sharpening, stabilizing and upsizing. Each image was run through multiple iterations using various combina-

tions to produce an image in which the bases/heads of the *Xenobalanus* were most visible. The Supplemental Material S-2 gives more details and examples. We then compared right to left sides of NZOP–005 to assess asymmetry in the distribution of the barnacles.

RESULTS

Pigmentation, rake marks & cookie cutter shark bite marks

NZOP–005 was photographed on six occasions between 2008 and 2019 (i.e., 4,090 days (or 11 years, 2 months, 12 days) apart; Table 1, Figs. 2–15, and see "Identification of the orca NZOP–005"
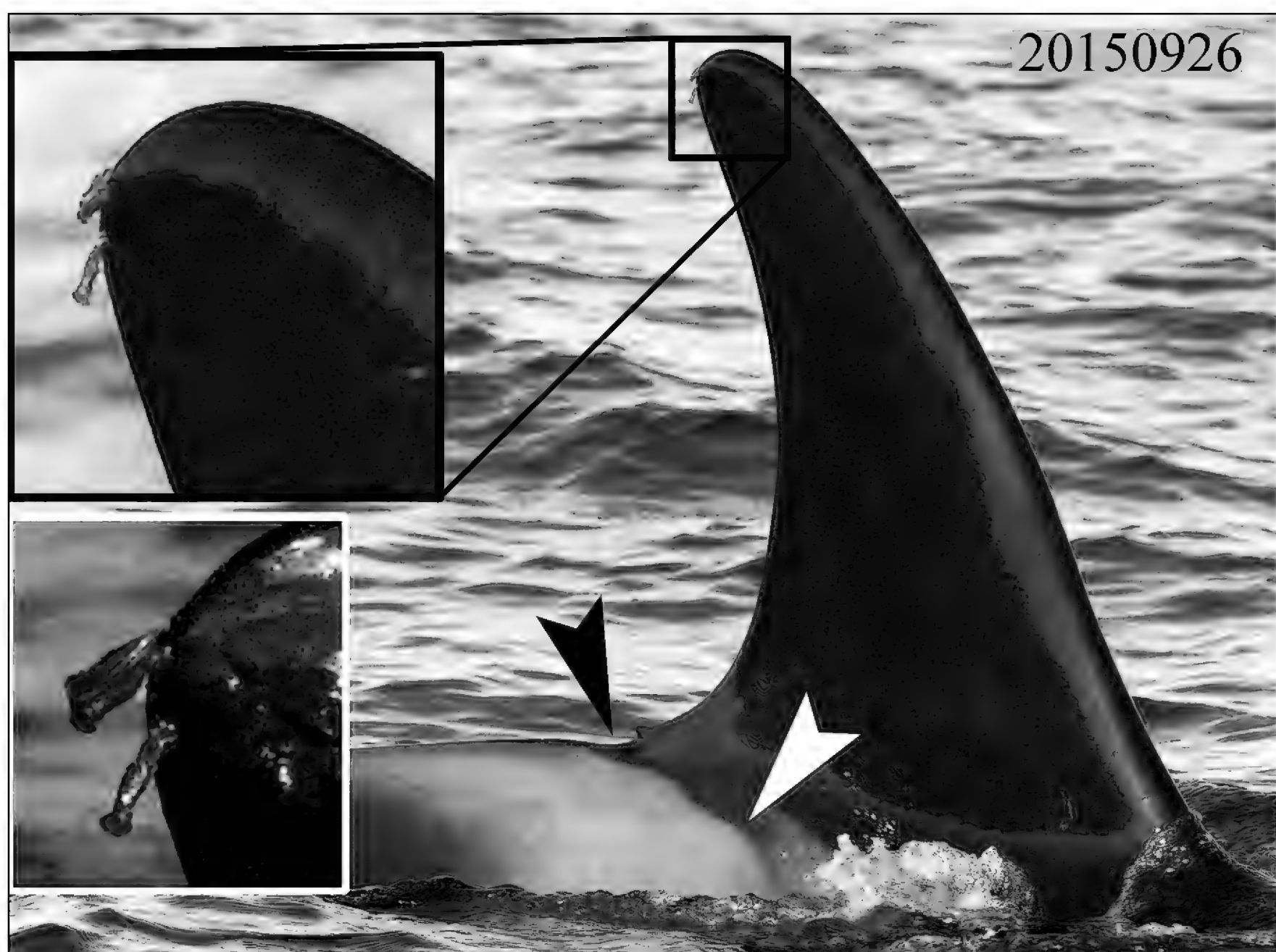
Figure 3. NZOP–005 photographed on 20150926, in the Hauraki Gulf, ~165 km to the south of her previous sighting. Three *Xenobalanus globicipitis* barnacles are on her dorsal fin (the third is partially obscured, top insert). The lower insert is from the same day, but a different photograph. The indent on her spinal ridge is visible (black arrow). The duration between the sighting in figure 2 and this sighting is 35 days. Also note the 'open' saddle patch (white arrow, see text for details). Photographs by INV.

for details. In the higher-resolution images of 2015 and 2019 (Figs. 5, 6) the band of four parallel rake marks were visible for at least 1,529 days (or 4 years, 2 months, 7 days) (Figs. 5, 6, white arrows). In the lower resolution image from 2008 (Fig. 4), the rake marks are possibly present (white arrow), but they cannot be confirmed, therefore that date was not included in the calculation.

The cookie cutter shark bite mark remained a consistent identifying feature across the years (Figs. 4–6, black arrows). An additional cookie cutter shark bite mark, lower down on her left saddle patch was visible for the same period of time (Figs. 4–6, circled). When NZOP–005 was photographed on 20191029, a third cookie cutter bite mark was visible on her left saddle patch (Fig. 6, insert). Both marks would also be classified as "SCAR", with the latter having a dark pigmented 'ring' around it (Fig. 6, insert).

Xenobalanus globicipitis

Although NZOP–005 was photo-identified for the first time in August 2008 in the Bay of Islands, Northland (record #1, Table 1, Fig. 7), the images from that encounter were of insufficient resolution to determine if *Xenobalanus* were present (Fig. 4). She was resighted just over one year and seven months later (in March 2010, record #2, Table 1, and see Visser et al. (2010)), within only a few kilometres of the first sighting and no *Xenobalanus* were photographed or noted. Next, NZOP–005 was photographed five and half years later (in August 2015, record #3, Table 1, Fig. 7), and at that point she was photographed with three *Xenobalanus* on her dorsal fin (Figs. 2, 5). This sighting was only ~17 km from the two previous sightings in the Bay of Islands). Subsequently, 35 days later (in September 2015, record #4, Table 1, Fig. 7) she was photographed to the south in the Hauraki Gulf, ~165 km from the Bay of Islands. On that day her tail flukes were first photographed and a small rounded notch was observed on her right fluke (Figs. 8, 9, blue arrows) along with a shallow section missing from the trailing edge (Figs. 8, 9, green arrows). At least 40 barnacles were visible along the trailing edge (> 26 on the right and >14 on the left fluke, Fig. 8, Table 1 and see Supplemental Material S-2).
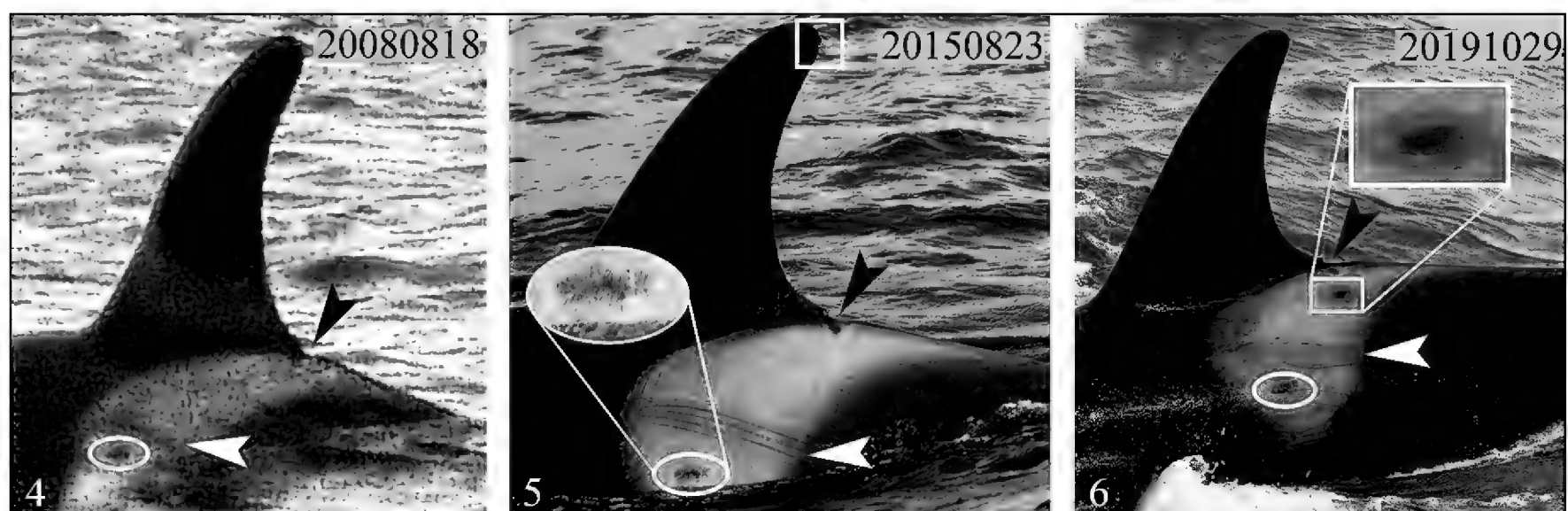
Figures 4–6. When NZOP–005 was first photographed on 20080818 (Fig. 4, left), although the resolution of the image was very low, when compared to subsequent Figs. 5, 6 it is possible to locate two cookie cutter shark bite marks (CCSBM); one on her spinal ridge (black arrow) and one low on her left saddle patch (circled). In figure 4 there is possibly a dark band of rake marks from conspecifics teeth (white arrow and see Figs. 5, 6, for placement). When next photographed on 20150823 (Fig. 5), the higher resolution image shows three *Xenobalanus globicipitis* on the tip of her dorsal fin (square, see also Fig. 2). The two CCSBM are still visible, with the typical 'puckering' of one these scars now visible (insert). The rake marks are clearly visible as four parallel darker pigmentation lines (white arrow). Four years, two months and seven days later, she was photographed on 20191029 (Fig. 6), and she had no barnacles on her dorsal fin. The two CCSBM remained visible (black arrow and circle, with the latter obscured slightly by the light reflection). She had acquired a new CCSBM on her left saddle patch with a dark ring of pigmentation surrounding it (insert). The original four rake marks had faded somewhat and she had acquired new rake marks below them (white arrow). The total duration that the spinal ridge and left saddle CCSBM (and potentially the rake marks), were documented was 4,090 days (i.e., 11 years, 2 months, 12 days), which exceeds the previous published record of 1,158 days (Dwyer & Visser, 2011). Photographs by Jochen Zaeschmar (left) HG (middle) and Auckland Whale and Dolphin Safari, Andy Light (right).

They were spread along nearly the entire span of both flukes, but in some places clustered tightly together along the trailing margin (Figs. 8–10). Due to that clustering, the number of barnacles estimated was conservative because tightly clustered individuals obscured conspecifics (see Supplemental Material S-2 for examples).

The following day (i.e., 20150927, record #5, Table 1, Fig. 7), she was resighted ~4.5 km to the east and underwater video was obtained using a HD pole-cam. As NZOP–005 made a number of passes close by the boat, both sides of her body as well as her tail flukes were documented. Again, numerous *Xenobalanus* were documented on the trailing edge of her tail flukes (Fig. 11) while on the distal edge of her right pectoral fin there were >22 (Fig. 12) and on the left >14 (Fig. 13).

We could see no barnacles on any other part of her body. Of note is that many of the *Xenobalanus* in the underwater images appear to have pale body colouring, however a comparison between the topside photographs showing the red-brown colouring of the body of the barnacles and the subsequent underwater video frames (with the paler colour), illustrate this is an artifact of colour absorption by the water. This is due to the light absorption and scattering properties of water whereby red light, which is a long-wavelength light and is the most affected, is reduced to ~1/3 of its intensity after just a metre's distance (Xinwei et al., 2015).

There was a noticeable difference in the number of *Xenobalanus* on her right and left sides (Table 1, Table 2, Figs. 8, 9, 11–13 and see Supplemental Material S-2). We could find only limited records in the literature where the numbers of *Xenobalanus* on both right and left were documented for individual cetaceans, of any species (Table 2).

The duration of attachment of *Xenobalanus*, as well as the potential settlement and/or rapid growth is suggested by records #3, #4 and #5, as although NZOP–005 was documented with three *Xenobalanus* on her dorsal fin during all three encounters, there were some discrepancies. In figure 14 the bases of the three barnacles are approximately equidistant from each other. However, although barnacles 'A' and 'B' may have remained attached between all three sightings, it is apparent that at least one barnacle (labelled as barnacle 'C' in figure 14), although present in August is no longer present in September, and instead, a scar remains (Fig. 15).
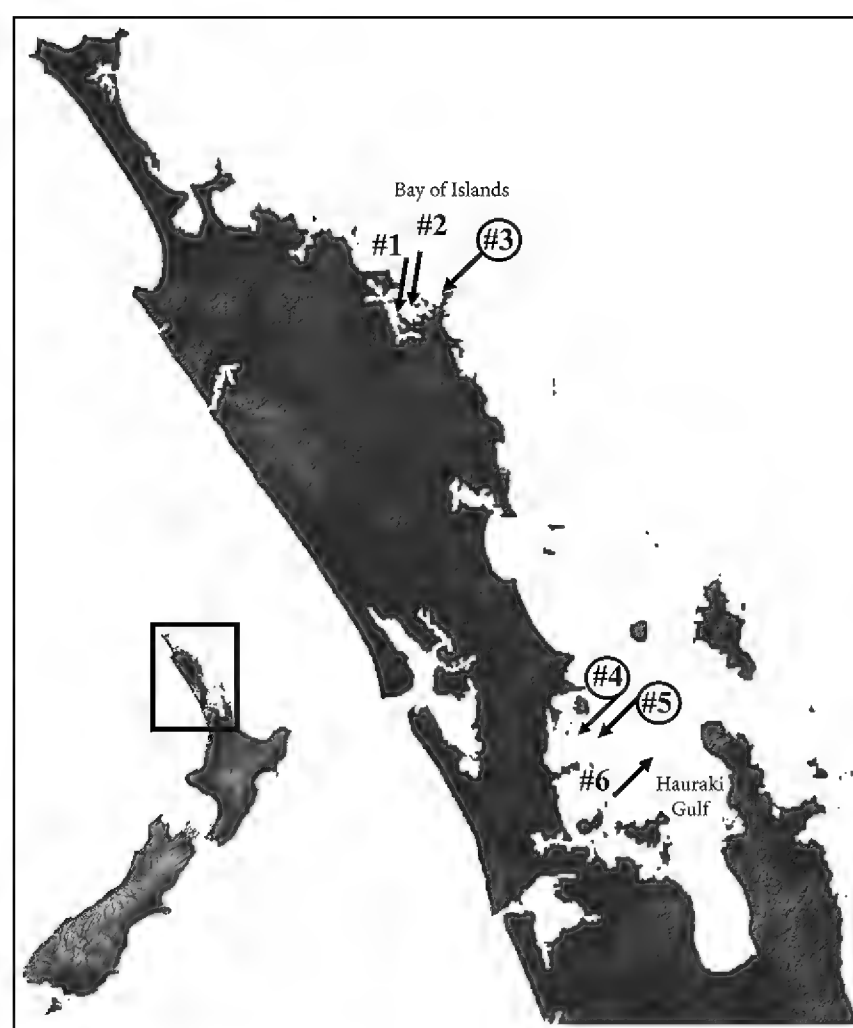
Figure 7. Sighting locations in Northland, New Zealand, for the female Pelagic ecotype orca (ID# NZOP-005), (see Table 1 for dates and further details). She was documented with *Xenobalanus* at three locations #3, #4 & # 5, circled. The distance between #3 at Cape Brett, Bay of Islands and #4 in the Hauraki Gulf was ~165 km (and 35 days apart), whilst the distance between #4 and #5 was ~4.5 km and one day.

Another barnacle (labelled '+1' in figure 15) has either become newly settled or it was of such a small size in August that it was not visible in the photograph and has since grown to be a size similar to its adjacent conspecifics. Although two other scenarios cannot be ruled out; (a) '+1' may instead be barnacle A or; (b) both A and '+1' are new recruits and have grown to this size in 36 days. Regardless, based on the proximity of these two *Xenobalanus*, at least one was not attached on 20150823. Therefore, four barnacles were attached, either sequentially or concurrently, at some point over the ~month-long period and, of those, two appear to have remained attached over the 36-day period.

After 20150927, NZOP–005 was not photographed again until 1,494 days later (i.e., 4 years, 1 month, 3 days) on 20191029 (record #6, Table 1, Fig. 7). Despite the duration between sightings, she was documented only ~23 km to the south of

the 2015 sightings, again in the Hauraki Gulf. When she was photographed during the 2019 encounter there were no *Xenobalanus* on her dorsal fin (Fig. 6).

There was no seasonal trend to the sightings of NZOP–005 as she was documented in three Austral seasons (i.e., not summer, Table 1) and the sightings of the *Xenobalanus* were only four weeks apart. The sea surface water temperature ranged from 14–21 °C (14–16 °C on the three days the *Xenobalanus* were documented) and depths from 18–43 m (25–40 m on days the *Xenobalanus* were documented) (Table 1).

DISCUSSION

Pigmentation, rake marks & cookie cutter shark bite marks

Although 'open' saddle patches have been documented on NZ Coastal and Antarctic orca ecotypes in NZ waters (Visser, 2000; Visser & Cooper, 2020b), they are not a typical pigmentation pattern in these waters. Between 1992–1997, 52 individuals from the NZ Coastal orca population were photographed on both the left and right sides (Orca Research Trust, unpublished data) and 49 of those

Record #, Date (yyyymmdd) Austral Season	Geographic Location	*Isistius* sp. bite mark(s) (left side)	*Xenobalanus globicipitis* (number / location on host)	Water Temp / Depth	Distance (direct line by sea from previous sighting)	Duration (since previous sighting – including end date)	Source
#1 20080818 Winter	Tapeka, Bay of Islands 35°14' S, 174°07' E	1, spinal ridge 1, lower saddle	Low resolution image, not possible to determine if present (Fig. 4)	14.4 °C 18 m	N/A	N/A	Photographed by Jochen R. Zaeschmar
#2 20100325 Autumn	South & East of the Black Rocks, Bay of Islands 35°13' S, 174°09' E	1, spinal ridge 1, lower saddle	0, on dorsal fin	21 °C 35 m	~ 3.7 km (to the north)	585 days or; 1 year, 7 months, 8 days	Photographed by Jochen R. Zaeschmar, Dolphin Explore (Tommy Hatwell) Visser et al., (2010)
#3 20150823 Winter	Cape Brett, Bay of Islands 35°10' S, 174°19' E	1, spinal ridge 1, lower saddle	3, near tip of dorsal fin (Figs. 2, 5)	16 °C 40 m	~ 17 km (to the east)	1,978 days or; 5 years, 4 months, 30 days	Photographed by INV & HG
#4 20150926 Spring	North of Whanga-paraoa Peninsula, Hauraki Gulf 36°32' S, 174°49' E	1, spinal ridge 1, lower saddle	3, near tip of dorsal fin (Fig. 3) >14, L tail fluke >26, R tail fluke (Fig. 8)	14 °C 25 m	~ 165 km (to the south) (@1738 hrs)	35 days or; 1 month, 4 days	Photographed by INV & HG
#5 20150927 Spring	North East of Whangaparaoa Peninsula, Hauraki Gulf 36°32' S, 174°52' E	1, spinal ridge 1, lower saddle	3, near tip of dorsal fin >22, R pectoral fin >14, L pectoral fin >26, R tail fluke >14, L tail fluke (total >79) (Figs. 2, 3,9, 11-13)	14 °C 30 m	~ 4.5 km (to the east) (@0032 hrs)	1 day	Photographed by INV & HG
#6 20191029 Spring	Hauraki Gulf 36°37' S, 174°06' E	1, spinal ridge 1, lower saddle, 1 upper saddle	0, on dorsal fin (Fig. 6)	16.5 °C 43 m	~23 km (to the south east)	1,494 days or; 4 years, 1 month, 3 days	Photographed by Auckland Whale and Dolphin Safari (Andy Light)

Table 1. Sighting dates and locations of NZOP–005, a female Pelagic ecotype orca (*Orcinus orca*), noting records of cookie cutter shark (*Isistius* sp.) bite marks (on her left side as this was documented in all sightings) and pseudo-stalked barnacles (*Xenobalanus globicipitis*), as well as the distance and duration between sightings. She was documented six times over a period of 4,090 days (or 11 years, 2 months, 12 days). Austral Seasons; Spring = September, October, November; Summer = December, January, February; Autumn = March, April, May; Winter = June, July, August. (http://www.bom.gov.au/climate/glossary/seasons.shtml).

had symmetrical saddle patches, while only three had asymmetrical saddle patches (Mäkeläinen, 1999). NZOP–005, a NZ Pelagic ecotype, exhibits an 'open' left saddle patch, but her right is 'smooth' (following the definitions given in Sugarman, 1984 and Baird & Stacey, 1988). Traditionally, orca identification catalogues tended to feature just the left side (e.g., see Bigg et al., 1987; Heise et al., 1991; Ford et al., 1994; Ford & Ellis, 1999), however, more recently both sides are published in catalogues (e.g., Denkinger & Alarcon, 2017) or only one side is published and the other is available within the database of the research project (e.g., Visser & Cooper, 2020b and the Center for Whale Research as cited in Visser & Mäkeläinen, 2000). To increase the probability of matching individuals, reducing mismatches and also minimising missed matches, as well as to document asymmetry in saddle patches, we recommend that photographs of both sides of individuals are collected.

The duration of visible rake marks on NZOP–005 is the first indicating longevity of rake marks for over four years. Conspecifics rake marks on orca are a poorly reported phenomenon with most descriptions being of limited nature (e.g., see Scheffer, 1969; Lockyer, 1979; Hoyt, 1984; Ford et al.,

1994). There is only one publication that reports 'prolific' rake marks on free-ranging orca and those included extensive and numerous marks in parallel rows of three or four over much of the body of two male orca (Visser, 1998). The behavioural cause for those rake marks, although attributed to conspecifics, was not established, but aggression is rarely reported in free-ranging populations (Towers et al., 2018). Yet it is prevalent in captivity, where aggression has been linked to rake marks, which can also be extensive (e.g., see Figs. 16–18 in Visser (2012), where more than 90 attacks were recorded in 77 hours of observations). The causative behaviour behind the rake marks on NZOP–005's saddle patch is undeterminable as the event during which they were formed was not witnessed.

Cookie cutter shark bite marks are not commonly documented on the NZ Coastal orca population, with the first being recorded in 2007. That bite was visible for 1,158 days (or 3 years, 2 months and 1 day) (Dwyer & Visser, 2011). The record for longevity of a cookie cutter bite mark being visible, is now held by NZOP–005 who exhibited two bite marks on 20080818 (i.e., on her spinal ridge and on her lower left saddle patch) which were still visible on 20191029 (i.e., 4,090 days (or 11 years, 2

Region	Species	Date	R fluke	L fluke	R pectoral	L pectoral	Dorsal fin	Total*	Source Information	Source
India	*Tursiops truncatus*	19850128	8	4	1	1	0	14	page 197 (text), page 198 (Fig. 1), page 199 (Table 1)	Rajaguru & Shantha (1992)
India	*Tursiops truncatus*	19980329	4	0	0	0	0	4	page 879 (text), page 880 (Fig. 2)	Karuppiah et al., (2004)
South Africa	*Orcinus orca*	Not given	5	2	9	5	ND	21	page 266 (text)	Best (2007)
South Africa	*Orcinus orca*	Not given	>6	2	ND	ND	ND	9	Their Supl. Fig. S2	Whitehead et al., (2014), S2 photo by Lloyd Edwards
New Zealand	*Orcinus orca*	20150927	26	14	22	14	3 (+1)**	79 (+1)**	Figs 2, 3, 6 & 8	This paper

Table 2. Comparison of *Xenobalanus globicipitis* attached on right (R) and (L) sides of cetaceans in the published literature (> 70 manuscripts were assessed for R and L details, however only four contained this type of data). Note; ND = Not documented, whereas '0' = documented as not present. * Numbers given by source, or total visible in photos, whilst also recognising that not all appendages may have been photographed/assessed. **between sightings, one *Xenobalanus* was shed and another (+1) grew to a similar size as its adjacent conspecifics, so although there were only ever three documented at any one time, there were four documented on the dorsal fin over time (see figures 14 and 15 and text for details).

months, 12 days) later). At that point she was also documented with a third cookie cutter bite mark in her left saddle patch. That mark had a distinctive dark ring of pigmentation around it, similar to one documented in Dwyer & Visser (2011, their Fig. 5b), which was 150 days post an open-wound state, suggesting that the third bite mark on NZOP–005 may have been relatively recently healed.

Xenobalanus globicipitis

Kane et al. (2008) provided a review of published and unpublished accounts of *Xenobalanus* on 34 cetacean species in 24 regions, however it contained no records of *Xenobalanus* on any cetacean species in NZ waters. Although Kane et al. (2008) included *Xenobalanus* on orca (with an emphasis on the Eastern Tropical Pacific region), a number of new records in the regions of Australia, Brazil, Canadian Arctic, Canary Islands, Galápagos Islands, Gibraltar, Guatemala Basin, Japan, Mexico, Peru and South Africa have since become available (see introduction for citations). We now add NZ (this paper, Fig. 1) and note that this is the first record of *Xenobalanus* on any cetacean species in NZ waters.

The earliest records of *Xenobalanus* on orca, globally, come from the Mediterranean area, where two orca were captured six years apart, in 1896 and 1902 (Richard & Neuville, 1897; Gruvel, 1920; Richard & Neuville, 1936). Records have been sporadic in the area since; e.g., Foote et al. (2011) stated that in their 1971–2008 study "*No* Isistius *wounds or* Xenobalanus *barnacles were seen on any individuals in more northerly waters* [than the Canary Islands] *including the Strait of Gibraltar.*". However, in contrast, the online catalogue of orca from the Strait of Gibraltar (CIRCE, 2020) includes a number of orca who have *Xenobalanus* on their dorsal fins and the dates those images were taken spans 2004–2013. A potential change in the distribution of *Xenobalanus* (or their hosts' dispersal/use of core areas) may have occurred multiple times in that area. Rappé & Van Waerebeek (1988) stated that "*it would appear that its* [Xenobalanus] *occurrence in the northeast Atlantic and Mediterranean is erratic, being separated by great lapses of time.*" Although early records are limited, an apparent trend of *Xenobalanus* being present in the late 1800's and early 1900's, absent during the 1970's

until approximately 2004, and present again more recently indicates support for this temporal distribution theory.

With respect to geographic distribution of orca from more contemporary times, they seasonally occupy the Strait of Gibraltar (e.g., see de Stephanis et al., 2008), whilst in the Mediterranean Sea they are considered 'visitors' (e.g., see Reeves & Notarbartolo de Sciara, 2006) which may be influencing our knowledge of both the host and the barnacle in those regions. Changes in spatial distribution may also be occurring in other regions. The potential of long-range movements by orca in the Canadian Arctic has been speculated (Matthews et al., 2020), as *Xenobalanus* have only recently been documented on the dorsal fins of some individuals who may be travelling to warmer waters. It is recognized that the loss of Arctic sea ice is causing a change in the sightings of orca in the Canadian Arctic (Higdon & Ferguson, 2009) including their 'pulses of advancement' into areas they have not previously been documented. Alternatively, it could be the *Xenobalanus* barnacles which have a changed distribution. For example, human assisted dispersal of larvae could create a rapid increase of sightings in an area, as suggested by Siciliano et al. (2020), i.e., the possibility that the recent occurrence and subsequent infestations on cetaceans of *Xenobalanus* off Brazil, originated from the conveyance of their larvae in vessel ballast water.

Worldwide, most records of *Xenobalanus* are from tropical and temperate regions and the barnacle is thought to be a warm-water species (Rappé & Van Waerebeek, 1988) based on the concentration of records given in Rajaguru & Shantha (1992) and Kane et al. (2008). However, in subtropical eastern Australia, Orams & Schuetze (1998) found that *Xenobalanus* presence on *Tursiops truncatus*, Montagu, 1821, was significantly less in summer (water temperatures of 24 °C) than when the water was 18 °C, indicating a thermal preference towards cooler temperatures. Over the last century the world has experienced an exponential rise in marine heatwaves affecting oceanic environments including shifts in marine species distribution (Oliver et al., 2018). In NZ, Pinkerton et al. (2019) report increasing sea surface temperatures "*with average rates of warming between +1.2 and +2.8 °C per decade (mean of +2.0 °C per decade)*". However, future climate change scenarios indicate a potential de-
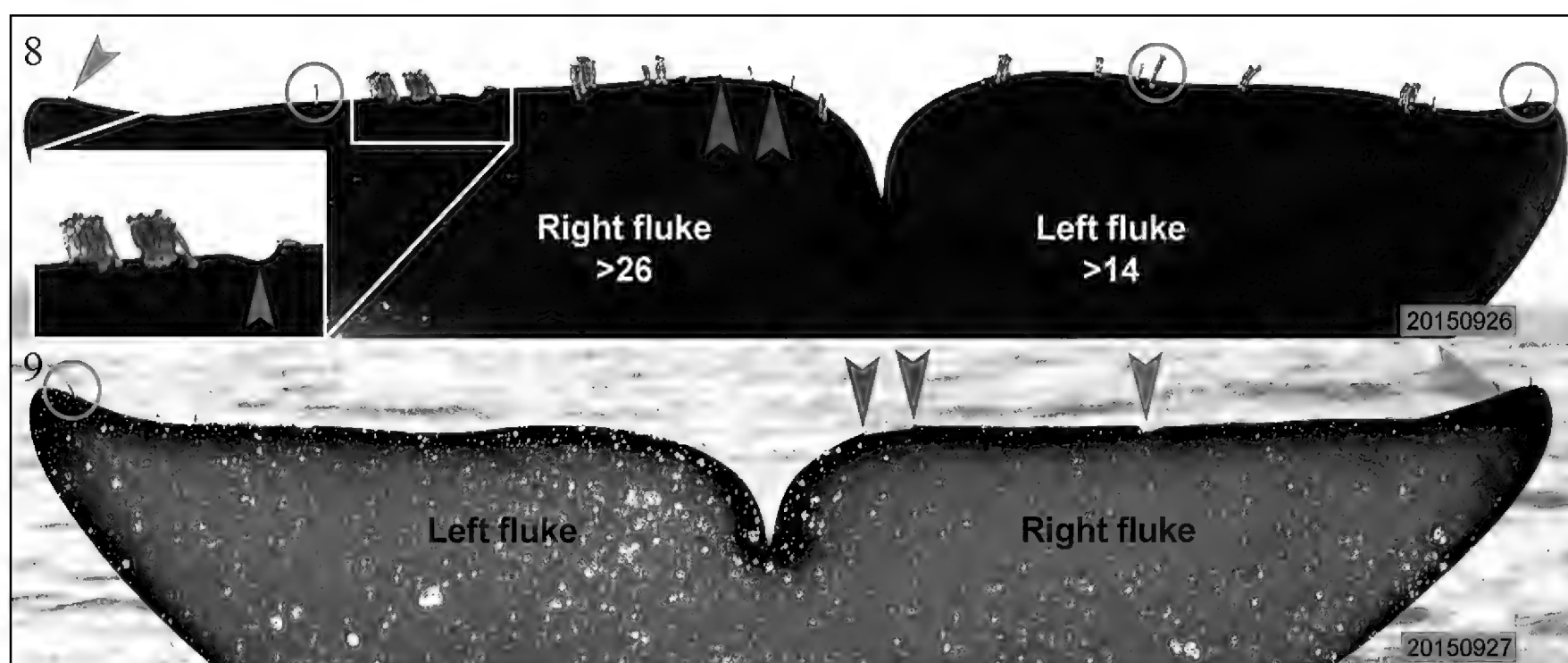
Figures 8, 9. On 20150926, the tail flukes of NZOP–005 were photographed for the first time (Fig. 8). There were at least 40 *Xenobalanus* (>26 on her right fluke and >14 on her left fluke, see S-2 for details regarding numbers). The distribution along the trailing edge extended nearly the whole length of each fluke with some settled as singles or spread out (brown circles) and others clustered tightly together (Fig. 8 insert, Fig. 10 and S-2). The close clustering obscured some barnacles, therefore the number of individuals was likely higher than indicated. Dorsal views of tail flukes do not typically show barnacles attached to the ventral side, unless they are forced into view via centrifugal force (e.g., brown arrow at left of figure 8 and then at right in figure 9). The blue arrows indicate a rounded notch in her right fluke while the green arrows indicate a missing shallow area which had 'hard corners' (also see Fig. 10). Although figure 9 was taken a day later than figure 8 and appears to have a significantly lower number of barnacles, comparison of this topside (ventral) image to the underwater (dorsal surface) fluke image in figure 11 (also taken on 20150927) establishes that the barnacles were still present. Photographs by HG (top), INV (bottom)

crease in water temperature for some locations (Long & Perrie, 2015). Rapid habitat change has been identified as putting all cirripedes at risk (Buckeridge, 2012) and as such, ocean thermal shifts and pH changes (e.g., ocean acidification) could be critical regarding *Xenobalanus* distribution, as they may be linked to growth and development, including mineral compositions of the shells as has been found for other barnacle species, e.g., Findlay et al. (2010) and Nardone et al. (2018). Although the pseudo-stalked *Xenobalanus* has reduced shells, there may still be implications for attachment to cetaceans, as the attachment plates are exposed above the epidermis of the host (Pugliese et al., 2012).

Certainly, the number of geographic regions where *Xenobalanus* have been documented has increased since the earlier reviews by Rajaguru & Shantha (1992) and Kane et al. (2008) where ten regions were listed collectively between 1896 (Richard & Neuville, 1897; Richard, 1936) and 2007 (Kane et al., 2008), i.e., in 111 years. We have now collated an additional 10 regions (including this

paper) in the 13 years since (i.e., 2007–2020). To reconcile such an extreme difference, we speculate what some potential causative factors may be and recognise that they may not be mutually exclusive. *Xenobalanus* logically could have gone undocumented on their hosts, or; they could have been under-reported or; more recently observer bias (such as access to better cameras, as well as an increase in dedicated on-water research projects) may have resulted in more records, or; a change in distribution of the hosts and/or the barnacles may result in less or more sightings. Nevertheless, without longer-term databases and robust documentation of the presence of *Xenobalanus* we may not be able to ascertain the driving force(s) behind these new regional records.

While other publications have noted *Xenobalanus* on orca when discussing photo-identification of orca (e.g., Fertl et al., 1996; Olson & Gerrodette, 2008; Pacheco et al., 2019), the duration that *Xenobalanus* remain on any individual host has not, to the best of our knowledge, been specifically documented. In Pacheco et al. (2019) a single adult

male orca was documented moving between the Galápagos Islands, Mexico and Peru and he is described as having "*between five to 10 coronulid barnacles (*Xenobalanus globicipitis*) attached*" on his tail flukes. Two of the photographs in that publication (their Fig. 2), show *Xenobalanus* and it is possible to establish the potential dates and locations where the images were taken from the data in the text. The duration between any of the dates, as well as the distance between the two locations, would far exceed the duration/distances given herein. However, the images in Pacheco et al. (2019) are of such low resolution that it is impossible to determine if the *Xenobalanus* are in the same

location on the tail flukes and, therefore, if they could potentially be the same individual barnacles or if they were subsequently recruited. Therefore, attachment duration cannot be determined from the data in that publication.

On NZOP–005, barnacle '+1' (Fig. 15) was not visible in August and yet it was a similar size to its conspecifics (barnacles A & B) 36 days later, suggesting a fast growth rate. Toth-Brown 2007, states, that "*These* [*Xenobalanus*] *larvae develop and settle once an appropriate host is found, and life cycles of 5–6 months have been reported (Van Waerebeek et al., 1993; Fertl, 2002)*". However, if the biological definition of the term "life cycle" is used, this

Figure 10. Close clustering obscures some barnacles, while others (e.g., a single barnacle circled in brown) are attached to the ventral side of the tail flukes and only become visible as the fluke is moved. Pale creamy-white basal plates can be seen in two *Xenobalanus* (white circle). At least one of the barnacles has its cirri extended beyond the 'hood' of the body (red circle). Due to the centrifugal force from the motion of the hosts' tail flukes, water previously adhering to the barnacles, was flung outwards (black arrows). The green arrows indicate a missing shallow area in the trailing edge of the tail fluke which had 'hard corners' (also see Figs. 8, 9). Photo post-processed using TopazLabs Stabilize AI and Gigapixel AI software. Photograph by INV.

implies that the barnacles live for 5–6 months. Fertl (2002: 75) actually makes no reference to this timeline other than to say *"The breeding season of barnacles that cling to whales is synchronous with that of the whales' breeding season."* where as Van Waerebeek et al. (1993: 167) state *"The clear six-month phase in peak occurrence suggests seasonally controlled swarming of nauplius larvae"*. In both instances these authors are referring to the breeding cycle, not the life cycle. Currently, the life-cycle (hatching to death) is unknown for this species.

Alternatively, although the barnacles labelled A and B are presumed to have remained attached during the 36-day period, it can't be ruled out that they may have been replaced by others in exactly the same locations on the dorsal fin. Toth-Brown & Hohn (2007) investigated *Xenobalanus* on 176 bottlenose dolphins (*Tursiops truncatus* Montagu 1821). They resighted some individual's multiple times in one or two years but they noted that *"the position of certain barnacles on the dorsal fin was slightly different from year to year (Fig. 3). This in-* *dicates that among years at least some barnacles had fallen off and were replaced with new ones."* and as such they too provide no duration data for any of the *Xenobalanus.*

The *Xenobalanus* we describe on NZOP–005 were typical with regards to their distribution on the body of the orca e.g., Fertl & Newman (2018) state "Xenobalanus globicipitus [sic]*, while worldwide in distribution, are almost always found on the trailing edges of the dorsal and pectoral fins and on the flukes of … cetacean species"*, although *Xenobalanus* are also occasionally located in other zones of the body of orca, such as at the base of the dorsal fin (Siciliano et al., 2020). Although Whitehead et al. (2014: fig. 1) provide images of orca off the coast of South Africa, it is not clear how many orca are depicted in the three photographs. Regardless, the distribution of the *Xenobalanus* on the dorsal fin and right pectoral fin for these South African animal(s) is similar to those on NZOP–005.

Carrillo et al. (2015) looked in detail at the distribution of *Xenobalanus* on 95 striped dolphins and found that they were more likely to be on the tail
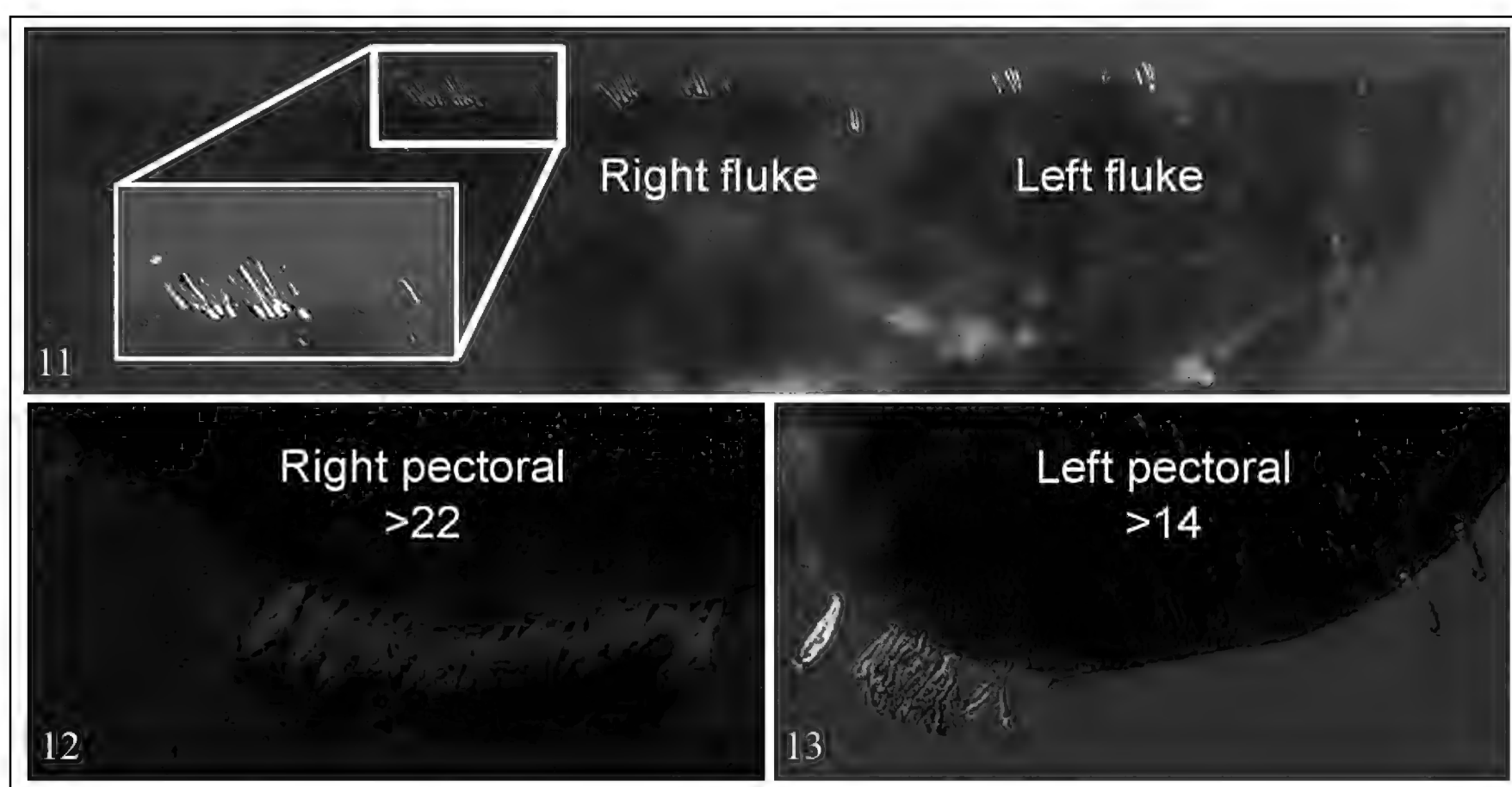
Figures 11–13. Frame grabs from underwater video taken on 20150927, show the *Xenobalanus globicipitis* on the tail flukes (Fig. 11), as well as the trailing edges of the pectoral fins (Figs. 12, 13) of the female orca NZOP–005. The barnacles appear lighter in colour as a result of the water filtering out visible red light (see text for details). This artifact is apparent when comparing the underwater images to the topside image of the flukes in figure 10, where the darker colour of the barnacles is visible. The approximate number of barnacles on the pectoral fins is indicated on the images (see S-2 for details). Frames from the video were post-processed using TopazLabs Stabilize AI and Gigapixel AI software. Insert is adjusted (contrast & brightness) to show notch. Images by HG and INV.

flukes than on the pectoral fins, with the lowest prevalence on the dorsal fins. When found on the tail flukes of the striped dolphins, the *Xenobalanus* were more likely to be on the dorsal surface than the ventral and more towards the centre of the tail than the distal ends. On NZOP–005 the lowest prevalence was also on her dorsal fin, followed by an increasing number on her pectoral fins and her tail flukes. And, like the dolphins, when *Xenobalanus* were on the tail flukes they were mostly on the dorsal surface, although they were spread over the whole span.

Although Carrillo et al. (2015) provide a very comprehensive analysis of the settlement patterns of *Xenobalanus*, they did not provide data on the prevalence of the barnacle's distribution on the right and left appendages. Likewise, Moreno-Colom et al. (2019) provide a detailed assessment of distribution of *Xenobalanus* on the tail flukes of dolphins paying particular attention to the dorsal and ventral surfaces, yet they provide no right and left side numbers. Barnacle species are often gregarious (Knight-Jones, 1953), including *Xenobalanus* who have been shown to aggregate and cluster together (i.e., the nearest neighbour distribution is not random, Moreno-Colom et al., 2019). In theory, barnacles settling onto the pectoral fins or tail flukes of a cetacean should be relatively evenly distributed on the right and left sides, if no other factors were at play. One might speculate that the higher number of *Xenobalanus* on the right side of the orca (see details for the three orca hosts in Table 2), may be a facet of favouring one side over the other.

The discrepancy of distribution between the number of *Xenobalanus* on the right and the left appendages of NZOP–005 may be typical for orca, as the (albeit limited) laterally distinguished data on *Xenobalanus* distribution on cetaceans, shows a similar trend of higher numbers on the right side (Table 2). At least eight species of cetaceans favour their right side for foraging (e.g., see Kaplan et al. (2019) and references therein). In contrast to that trend is one study on Indo-Pacific bottlenose dolphins (*Tursiops aduncus* Ehrenberg, 1832) off Japan which showed significant left-side bias when using a flipper for rubbing a conspecific (Sakai M. et al., 2006).

At least some orca show lateral asymmetry when using their pectoral fins and typically favour using their right side (Giljova et al., 2016), they show lateralization for the spatial relationships between mother and infant which also favours the

right side (Karenina et al., 2013) and during lunging (when feeding) the orca again show a favouring of the right side (Karenina et al., 2015). Such lateralization likely creates differential water flows over the appendages, potentially influencing settlement and/or feeding and/or growth of *Xenobalanus*.

Although little is known about the early life-history stages of *Xenobalanus*, water flow has been described as having an impact on settlement success of *Xenobalanus* (Wethey et al., 1988; Carrillo et al., 2015; Moreno-Colom et al., 2019) and the *"larval settlement on marine mammals can be considered an extreme lifestyle as the hosts are fast-swimming and migrate over great distances in the open oceans, but also because they slough the outermost, non-living cell layer of the epidermis up to several times a day"* (Dreyer et al., 2020).

The number of *Xenobalanus* documented on NZOP–005 (*n*=>79), were more than 3.5 times higher than any other orca; (*n*=22) in Canadian Arctic; (*n*=21) in South Africa. Yet the NZ *Xenobalanus* were also clearly transitory in nature (i.e., not present in 2010 and 2019 but present between, in 2015). With so few datapoints during the 11+ years it is not possible to know the full duration of attachment and what influences recruitment and/or separation from the host. One possibility is water temperature, as Dreyer et al. (2020) hatched *Xenobalanus* larvae at 28 °C and grew them to the cyprid stage in approximately eight days. NZOP–005 was not documented in summer and the sea surface water temperatures, when she was hosting *Xenobalanus*, were 14 °C–16.5 °C (Table 1). Van Waerebeek et al. (1993) noted a seasonal trend in the incidence rates of *Xenobalanus* on dusky dolphins (*Lagenorhynchus obscurus* Gray, 1828), from Peru, where August, September and October showed a downward trend, with the latter two being significantly lower than the peak months of May–July. They attributed these trends to a six-month phase of *"seasonally controlled swarming nauplius larvae"*. In NZ, the apparent settlement and rapid growth of barnacle '+1' on an orca between August and September, when the water was relatively cold, is suggestive that at least some settlement occurred and/or criteria for growth of *Xenobalanus* was met during the winter-spring seasons. And, although no measurements of the *Xenobalanus* were possible, when compared to the literature, the relative size and the morphology of most of the *Xenobalanus* on NZOP–005 suggests that they were near sexual
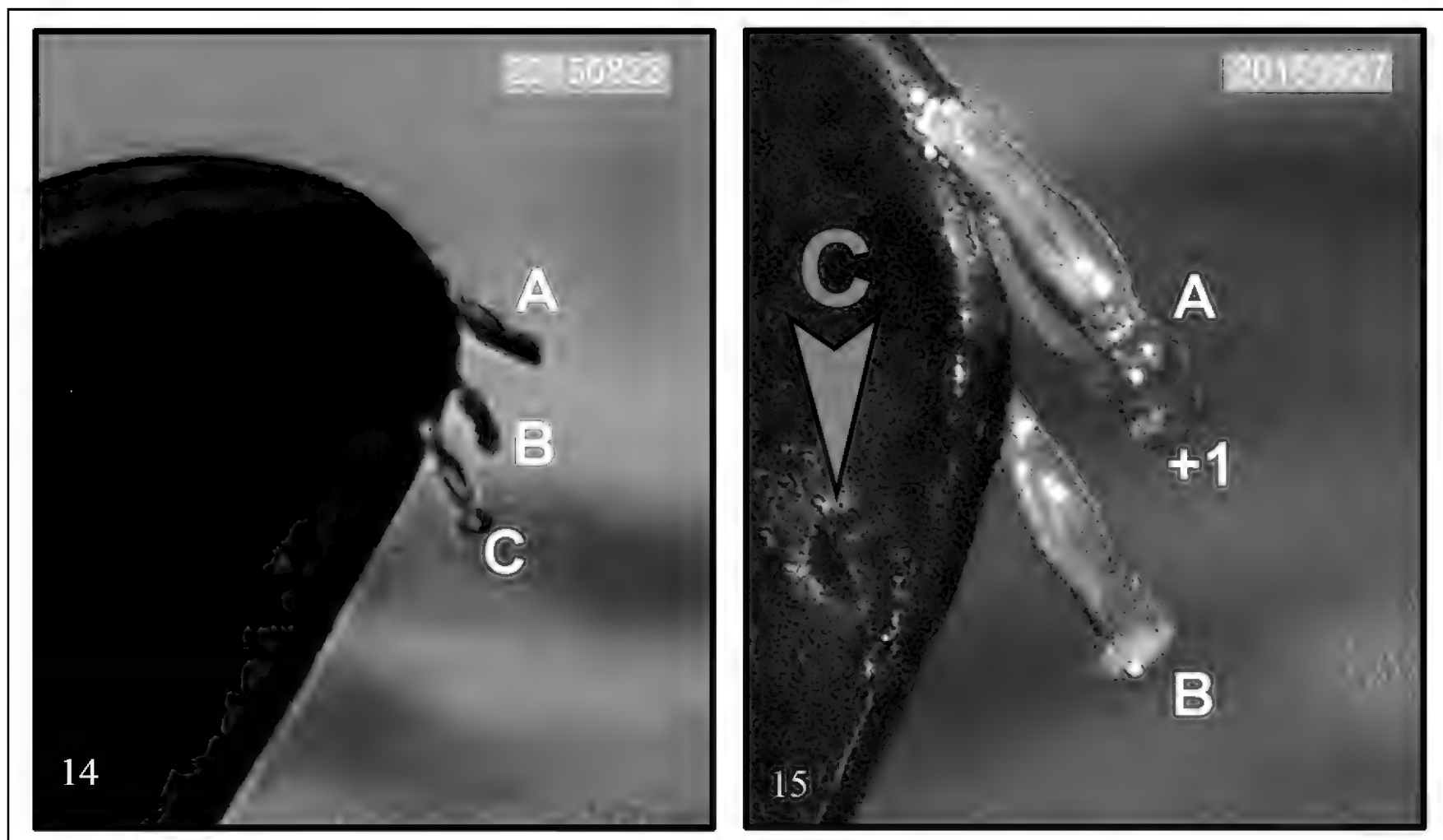
Figures 14, 15. The left side of the dorsal fin of NZOP–005, 36 days apart as indicated by the dates in yyyymmdd format on figures 14, 15. In figure 14, the three barnacles are approximately equidistant from each other. In figure 15, what is presumed to be barnacle A is attached on the edge of the dorsal fin, between the left and right sides, with another barnacle '+1' tucked in directly under it (although it cannot be ruled out that this may instead be barnacle A). Barnacle B is not attached to the edge, but is rather on the right side of the dorsal fin. Barnacle C is clearly visible in figure 14 and its attachment point is on the left side of the fin. However, in figure 15 barnacle "C' is no longer present and instead, a scar remains (arrow). Although it is not possible to ascertain which of the two upper-most barnacles in image Fig. 15 corresponds to barnacle A, it is clear that an additional (similar sized barnacle) is now present. The lighter colouring of the barnacles in Fig. 15, particularly when compared to those in figure 7, is likely an artifact of the light conditions at the moment the barnacles were photographed. Both photos were post-processed using TopazLabs Stabilize AI and Gigapixel AI software. Photographs by HG (14) and INV (15).

maturity or possibly adults (although there were some smaller (in length and girth) individuals present as well, see Figs. 8, 10). Given that the *Xenobalanus* appeared to be semi- to fully mature when they were first photographed on 20150823, it is logical that they would have been attached to the orca prior to this date, therefore the 36 days documented between these two events is the minimum duration that the barnacles were attached.

CONCLUSIONS

Although the evidence presented herein is derived from just one female Pelagic ecotype orca, the limited information is compelling. The long-term visibility of the rake marks on NZOP–005 helps confirm them as a valuable tool in identifying indi-

viduals, but it would be of benefit to have more data on their provenance in terms of the social/aggression interactions of conspecifics or inter-ecotype encounters.

The duration of the cookie cutter shark bite marks for more than 11 years gives weight to them being used as identification marks for individual recognition as well as biological tags for long-term periods and for distinguishing between ecotypes.

This first documentation specific to the duration (and colonisation/or growth) of the barnacle *Xenobalanus globicipitis* provides us with a baseline, but it also clearly illustrates a gap in our knowledge about the longevity of *Xenobalanus* and the driving forces behind their recruitment onto (and duration on) orca and what triggers their displacement. Furthermore, it also highlights that the use of *Xenobalanus* as population markers should be ap-

plied in a cautionary manner and where possible used in combination with other population indicators/markers (such as behaviour, pigmentation, cookie cutter shark bite marks etc). If used for individual identification of any cetacean, *Xenobalanus* should be well documented by photographs/video to ensure that their numbers are accurately verified, as well as to monitor the potentially transitional nature of the barnacles over time.

Given the 'loss' of barnacle C and the addition of barnacle '+1', using *Xenobalanus* for individual identification may be best suited to consecutive days/weeks where resightings are frequent. A similar recommendation was made, based on the seasonal occurrence of the barnacle on dusky dolphins, by Van Waerebeek et al. (1993) who suggested they "*can serve as a sensitive ecological marker on a small temporal scale, i.e. months…*". Longer-term monitoring of *Xenobalanus* will also help establish how robust they are as biological tags, including potential changes in distribution of the barnacles themselves (Siciliano et al., 2020).

Regardless, as is so often the case in the field of marine mammal biology (and in this instance also *Xenobalanus*), further documentation and reporting of similar scenarios, anomalies and even common place events, will help us to better understand the complexities of these species. For example, data regarding cookie cutter shark bite marks, which are rare on some ecotypes and common on others (Dwyer & Visser, 2011; Visser & Cooper, 2020b, 2020a), may help better define the ecotypes and their distribution. If bite marks appear more prevalently on an ecotype when they were previously uncommon, this may indicate a shift in the orca's habitat use (or that of *Isistius* sp.). As such, we encourage researchers to publish sightings of cookie cutter shark bite marks (and *Xenobalanus*), including historic data which could also help establish long-term trends, such as seen in here for the cookie cutter shark bite mark, or establish range extensions, such as seen here for the *Xenobalanus*.

With the consequences of climate change already influencing orca (Matthews et al., 2020), as well as other species of cetaceans (MacLeod et al., 2005; MacLeod, 2009), long-term impacts are likely and biological tags may be early indicators that changes are occurring. We therefore encourage management authorities to include in their action plans monitoring of both of these biological

tags (as well as other potential markers), not only in field research, but also when cetaceans strand/die. The limited published data on these two indicator species illustrates that all too often they are perhaps overlooked or not considered of value.

Additionally, when implementing action plans, management authorities should ensure they consider climate change and prioritise aspects that can have conservation benefits for cetaceans, given the dynamic climate framework and the consequences cetaceans will face from its impacts.

We also strongly promote the recognition of various orca ecotypes as separate stocks. These will require separate management plans as has been done for Bigg's (Transient) (DFO, 2007), Offshore (DFO, 2009) and Resident ecotypes (DFO, 2017) in Canada. Given that we can already differentiate between a number of orca ecotypes by using features such as the biological tags described herein, it would be remiss of management authorities to not conduct due diligence and create separate plans, as these could have real-world consequences for the animals in question. At the extreme, if appropriate management protocols are not implemented expeditiously, an orca ecotype, particularly one with a small population size, could go extinct before it was even properly recognised.

SUPPLEMENTAL MATERIAL

S-1. *Xenobalanus* in manuscripts from Richard & Neuville (1896), Gruvel (1920), Richard (1936) and Richard & Neuville (1936), with discrepancies in citations of these records.

S-2. Details and counts of *Xenobalanus* for each appendage of orca NZOP–005.

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Supplemental Material S-1. *Xenobalanus* **in manuscripts from Richard & Neuville (1896), Gruvel (1920), Richard (1936) and Richard & Neuville (1936), with discrepancies in citations of these records.**

During our review of records of orca hosting *Xenobalanus globicipitis* (hereafter referred to as *Xenobalanus*), we noticed some discrepancies/anomalies regarding three records and how they are cited. The unravelling of the issues should help to prevent further replication of the misinterpretation of the original data.
We use YYYYMMDD format to avoid complications between North American and other date formats.

1. 'Monaco'

On **18960527,** off the coast of Monaco two orca were harpooned and killed. This was first reported by **Richard & Neuville (1897)** in the scientific journal *Mémoires de la Société Zoologique de France*, volume X, pages 100-109.

Richard & Neuville (1897) on pages 105-108, give the date and the location as Station 638. The text also states the capture was *"off the coast from Monaco"*. The authors give the longitude as 4°57'45" E (which appears to be the departure point (Fos-ser-Mer / Port-de-Boc) rather than the capture point [7°18' E], see Richard & Neuville (1936) for capture location & Table S-1.1 for comparisons & Fig. S-1.1 for map).
Richard & Neuville (1897) mention that the orca was harpooned and give the total length measurement of the two females. They also mention *Xenobalanus* on the pectoral fins and tail flukes of the larger of the two orca.

There is one photograph of a dead female orca lying on her right side, showing her ventral surfaces and a rope around her tail (see last row of Table S-1.1).
This 1896 record is often erroneously attributed to other authors such as Richard (1936), see below for details.

Gruvel (1920), page 55 has the heading Genre **Xenobalanus**, Steenstrup 1951 [sic], and *"Xenobalanus globicipitis* Steenstrup*"*. On page 56 Gruvel (1920) mentioned the **18960527** event, but gives only these basic details; *"Expedition of 1896: Stn.* [station] *638 on an orca (Orca gladiator)"* [translated'] (i.e., he refers to the Richard & Neuville (1897) record, but no description is given of the location, other than mention of the station number).
Gruvel (1920) is sometimes listed as 'Mediterranean' in subsequent citations. Additionally, and erroneously, Gruvel (1920) is at times cited as the source publication for the 18960527 record (however, see below regarding the 19020722 record off Spain).

Richard (1936) is a volume comprised of various sections / chapters / publications, some of which have been previously published. Jules Richard is the Editor of the volume. This volume is often cited as the source for the 18960527 record. While in and of itself this isn't 100% incorrect, as a reprint of the Richard & Neuville (1897) manuscript is contained inside (albeit modified, see below and see Table S-1.1), it isn't 100% precise either. Furthermore, in order to be accurate, the

authors should be cited as Richard & Neuville (1936), not solely Richard (1936).

Richard & Neuville (1936) is a reprint of the Richard & Neuville (1897) scientific publication. Immediately following the title in the 1936 version is an *, which leads to a footnote giving an abbreviation of the *Journal Mémoires de la Société Zoologique de France*, the volume number (10) and the year; ("*Mém. Soc. Zool. De France, x, 1897*"), i.e., making reference to the original manuscript being published in 1897. However, we note that in this volume the text is changed somewhat (including adding in the 'Spain' ('Gibraltar') record as described below).

Richard & Neuville (1936) provide identical information in their pages 13-14 as the original (1897) publication, with the exception of correcting a longitude (see Table S-1.1). From that point forward there is additional data regarding the 1896052 record that was not included in the Richard & Neuville (1897) version. Richard & Neuville (1936) add that the animals were harpooned in the morning and details of the behaviour of the mother to her injured offspring. They also provided a detailed table of measurements of each of the two dead females (the mother and her presumed offspring). They note that some organs were preserved in salt.

2. 'Spain' ('Gibraltar')

On **19020722** off the coast of Spain an orca was harpooned and killed. This was first described by **Gruvel (1920)**, who only gives these basic details. "*Expedition of 1902. Stn.* [station] *1267 on an orca (Orca gladiator)*" [translated'] (i.e., there is no mention made of the location other than the station number). The 19020722 record appears to be linked to **Gruvel (1902)** (see Table S-1.3),

however we could find no details of either an orca capture nor records of *Xenobalanus* on an orca in the Gruvel (1902) record and, as such, we believe that the Gruvel (1920) publication is therefore the original source of information for the 19020722 record.

Richard & Neuville (1936) provide more data regarding the same **19020722** record. These authors mention 'near Gibraltar'. However, more accurately, from the latitude and longitude we can ascertain that the capture was approximately 30 km to the east of Gibraltar, and approximately 6.5 km off the coast of La Chullera. It was therefore off the coast of Spain (See Fig S-1.1) although it is often cited erroneously as a record from 'Gibraltar'.

Of note is that although the Richard & Neuville (1936) has an asterisk by the title, with a footnote that indicates the manuscript is a reprint of their 1897 manuscript (see section 1, above), it is not an exact replica. Rather it is modified to include this Spanish record (along with records of other cetacean species captured) (see Table S-1.1 for orca specific details). Additionally, the longitude in the text has been changed from the 1897 publication, to reflect the capture point, rather than the presumed departure point.

HOW HAVE THESE RECORDS BEEN CITED?

We note that authors, when discussing *Xenobalanus* on orca, typically do not cite Richard & Neuville (1897). In fact, we could find no recent publications that mention the 1897 publication at all, despite discussing the record from 18960527.

Instead, some cite Gruvel (1920) solely, or they may treat Gruvel (1920) and Richard & Neuville (1936) as if they were each an original record and overlook that both publications are referring to the same two records (e.g., Rajaguru & Shantha (1992), in their Table 1, list '*Mediterranean*' for Gruvel (1920) and '*Gibraltar*' for Richard (1936) – i.e., they also have the location incorrect).

Or, authors may conflate the two (e.g., Whitehead et al. (2014) "*in the Mediterranean (Gruvel 1920; Richard 1936)*"), or authors may cite Gruvel (1920) without any reference to location, just host species (e.g., Nilsson-Cantell, 1930 "*Orcinus orca (acc. To Gruvel, 1920)*").

Although we too list Gruvel (1920), that is done because it was the first published record of the capture of the La Chullera Spanish (aka erroneously referred to as the 'Gibraltar') orca, from which Richard & Neuville (1936) later describes the *Xenobalanus*.

And, although some authors conflate the two La Chullera and Monaco records into the 'Mediterranean Sea', we keep them separate (and therefore indicate each as separate 'regions' in Fig. 1 in our manuscript), because of the geographical distance of ~1,500 km between Monaco and La Chullera (i.e., a similar distance between the Galápagos Island and Costa Rica (~1,200 km) and more than the distance between the Canary Islands and Gibraltar (~1,000 km) and such a distance suggests both oceanographic and regionally biological differences are likely occurring between Monaco and Spain.
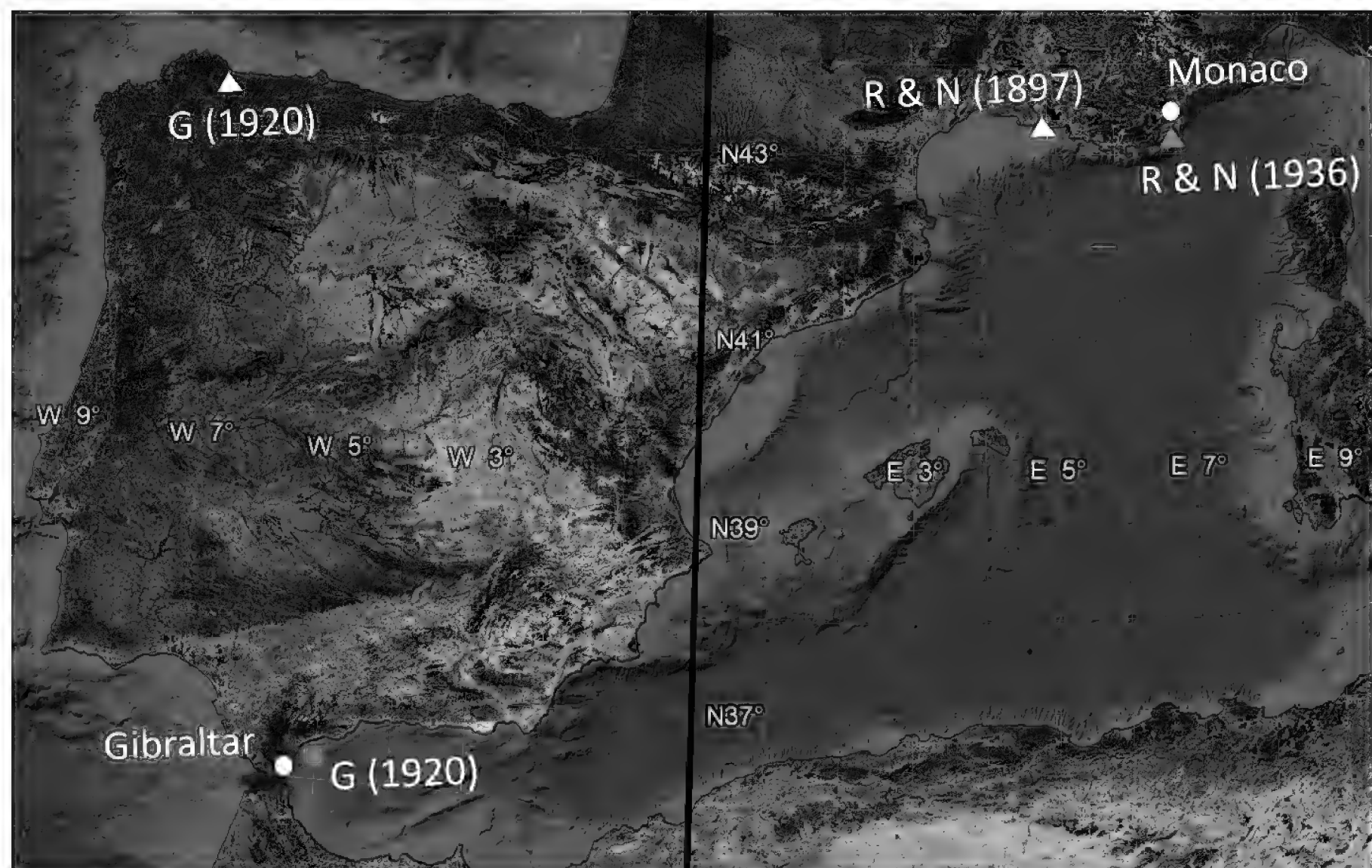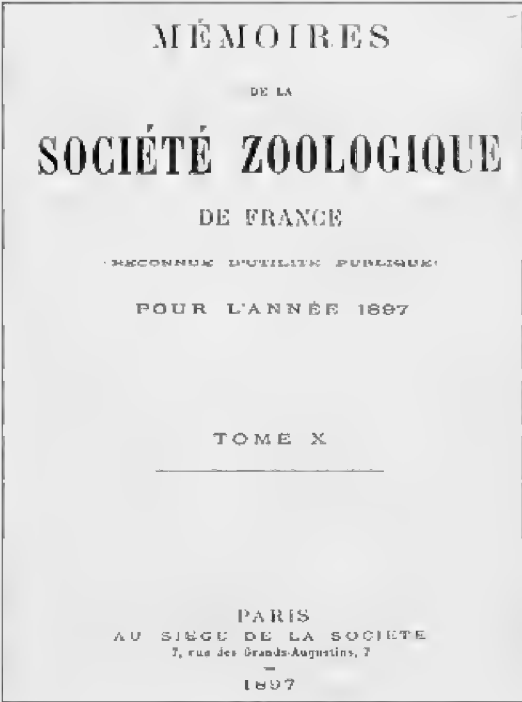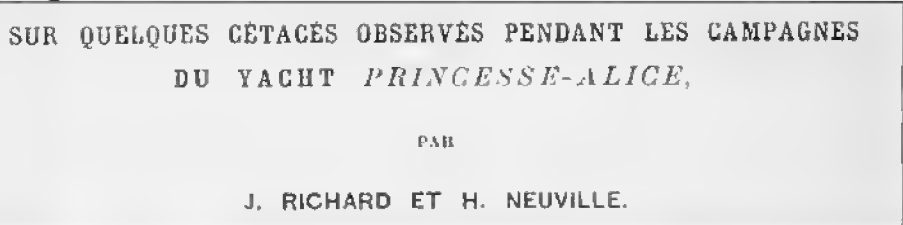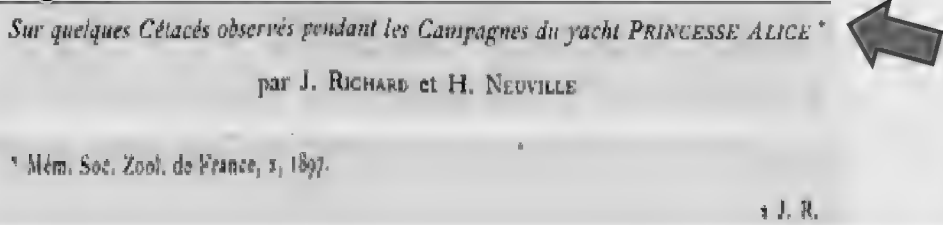
Figure S-1.1. Places referred to in the Richard & Neuville (1897) (**R & N (1897)**; Gruvel (1920), (**G (1920)**); and Richard & Neuville (1936), (**R & N (1936)**). The **18960527** record was captured off **Monaco** (red triangle), whilst the **19020722** was captured approximately 6.5 km off the coast of **La Chullera, Spain** (red square). Of note is that the latter is often referred to as captured off Gibraltar, however it was 30 km from Gibraltar and therefore well inside Spanish waters.

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Gruvel J.A. 1920. Cirrhipedes provenant des campagnes scientifiques de S.A.S.le Prince de Monaco (1885-1913). In; Résultas des Campagnes Scientifiques accomplies sur son yacht par Albert ler, Prince Souverain de Monaco. [Cirripeds from the scientific campaigns of HSH the Prince of Monaco (1885–1913). In; Results of scientific campaigns compiled on his Yacht by Albert 1st Prince Sovereign of Monaco. Published under his direction with the assistance of M. Jules Richard, Doctor of Sciences, in charge of Zoological works on board. Volume 53]. Monaco. 1-88 pp.

Richard J. & Neuville H. 1897. Sur quelques Cétacés observés pendant les Campagnes du yacht Princesse Alice. Mémoires de la Société Zoologique de France. X:100-109.

Richard J. & Neuville H. 1936. Documents sur les cétacés et pinnipeds des croisieres du Prince Albert 1er de Monaco réunis par Jules Richard [Documents on cetaceans and pinnipeds from the campaigns of Prince Albert 1st of Monaco collected by Jules Richard. In; Results of scientific campaigns carried out on his yacht by Albert 1st Prince Sovereign of Monaco. Volume 94]. In: Richard J, Editor. Résultats des campagnes scientifiques accomplies sur son yacht par Albert 1er Prince Souverain de Monaco Fascicule XCIV. Gouvernement á Monaco, Monaco, 34-71.

Table S-1.1. Comparing **Richard & Neuville (1897)** *verses* **Richard & Neuville (1936)** shows that the (1897) record should take precedence for the 1896 orca capture and therefore also the *Xenobalanus globicipitis* record. The two publications differ in that the 1897 publication was originally published in a scientific journal whilst the 1936 publication was a reprint as a chapter in a volume (report/book) **AND** there is reference to another orca capture on 19020722 (La Chullera Spain, often erroneously cited as Gibraltar). We present comparisons between the two publications to clarify that for the 18960527 orca record (Monaco), the 1897 version should be cited as the original source. For the 19020722 orca record (i.e., La Chullera, Spain), Gruvel (1920) should be cited as the original record of the capture of the orca[1]. Comments in ["*square brackets and italics*"] are translations. Comments in [square brackets & no italics] are additional information, such as clarification of a point. Underlined is different or new.

Richard & Neuville (1897) [On some cetaceans observed during the campaigns of the Princess-Alice yacht]	Richard & Neuville (1936) [On some cetaceans observed during the campaigns of the Princess-Alice yacht]	
1897 (scientific journal publication)	**1936** (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
Cover Journal Mémoires de la Société Zoologique de France Scientific Journal ["*Memoirs of the Zoological Society of France*"] MÉMOIRES DE LA SOCIÉTÉ ZOOLOGIQUE DE FRANCE RECONNUE D'UTILITÉ PUBLIQUE POUR L'ANNÉE 1897 TOME X PARIS AU SIÈGE DE LA SOCIÉTÉ 7, rue des Grands-Augustins, 7 1897	**Cover** ["*Results of scientific campaigns carried out on his yacht by Albert 1st sovereign Prince of Monaco*"] RÉSULTATS DES CAMPAGNES SCIENTIFIQUES ACCOMPLIES SUR SON YACHT PAR ALBERT Iᵉʳ PRINCE SOUVERAIN DE MONACO PUBLIÉS SOUS SA DIRECTION M. JULES RICHARD FASCICULE LIII Par A. GRUVEL IMPRIMERIE DE MONACO 1920	**OVERVIEW** **1897.** Scientific manuscript. Includes orca capture off **Monaco** on **18960527**. **1936.** Chapter. Reprint of 1897 manuscript, with correction of location (longitude) and additional information on the orca capture off **Monaco** on **18960527**. Additional orca record off **La Chullera, Spain** on **19020722**.
Page # 100 SUR QUELQUES CÉTACÉS OBSERVÉS PENDANT LES CAMPAGNES DU YACHT *PRINCESSE-ALICE*, PAR J. RICHARD ET H. NEUVILLE.	Page 11 Sur quelques Cétacés observés pendant les Campagnes du yacht *PRINCESSE ALICE* * par J. RICHARD et H. NEUVILLE * Mém. Soc. Zool. de France, x, 1897. ⸺ J. R.	The **1936** chapter title has an * next to it (arrow) and a footnote referring to the **1897** publication in *Mémoires de la Société Zoologique de France*. Otherwise, titles and authors are identical.

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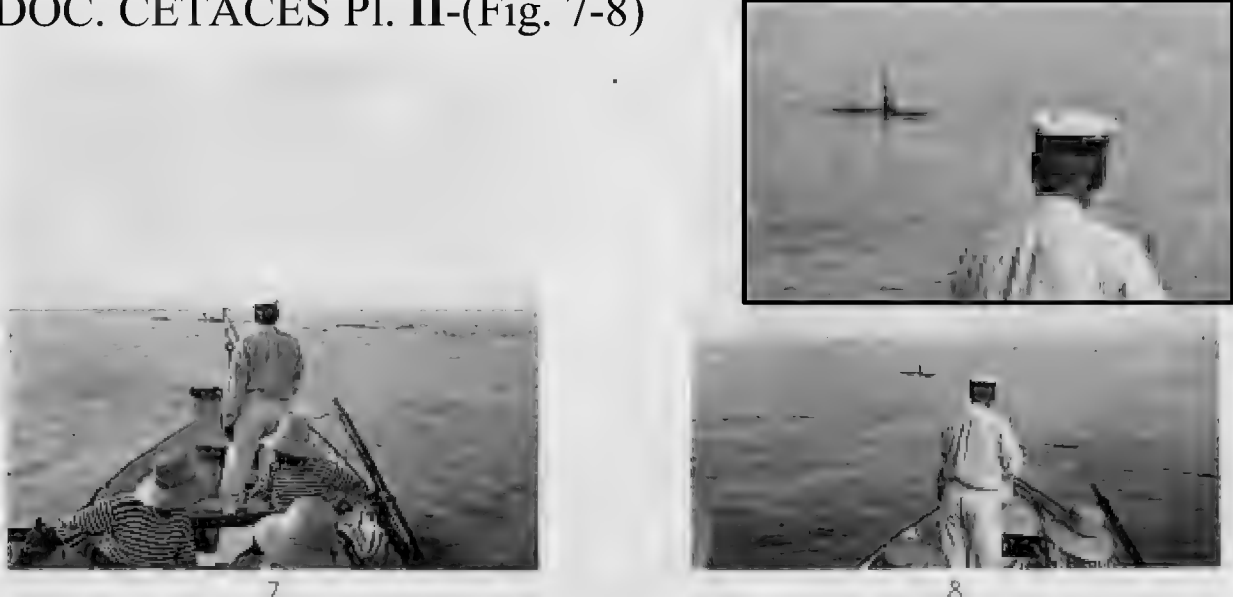
[1] See Table S-1.2 for details regarding Gruvel (1920)

1897 (scientific journal publication)	**1936** (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
Page # 105	Page # 13	**1897** publication gives the longitude as 4°57'45" E for the location (which appears to be the departure point; Fos-ser-Mer / Port-de-Bouc). **1936** reprint gives 7°18' E (which appears to be the correct capture location; off the coast of Monaco). **Otherwise, the text is identical** ["***Two female*** *killer whales were harpooned on May 27, 1896, off the coast from Monaco (Station 638), at 43°26' 20" lat. N,* <u>*4°57' 45" long. E.*</u> *A third one was wounded, but could not be captured. Of the first two individuals, one measured **5m 90**, and the other **4m 10** in total length*"].
Page # 106/107	Page # 14	**Text is identical** **1897** **1936** ["*As parasites, we have to report several* **Xenobalanus** *which were attached to the tail and pectoral of the large Orca*"]
Page # 107 **[MANUSCRIPT TEXT ENDS]** [SEE BELOW FOR PLATE from 1897]	Page #14	**Text is identical** **1897** **1936** ["*While the Grampus has been reported quite a number of times in the Mediterranean. The Orca has been so much more rarely (2), so the capture of two Orcas, near Monaco, is of great interest.*"] [note: the (2) is not clear with regards to its meaning, it may be that they have sighted them only twice].

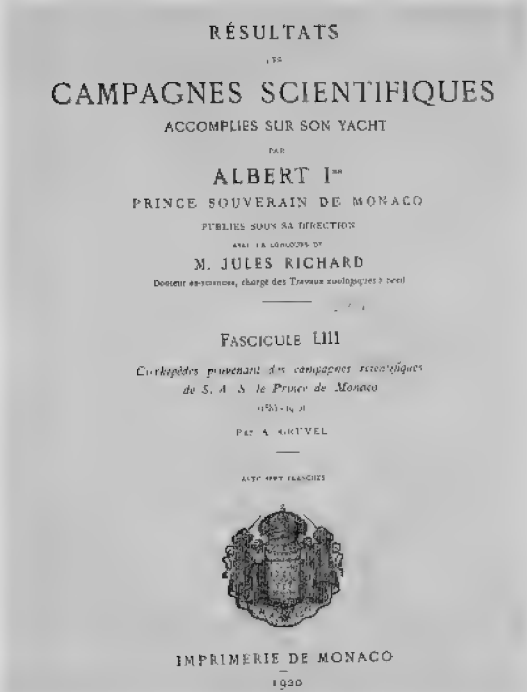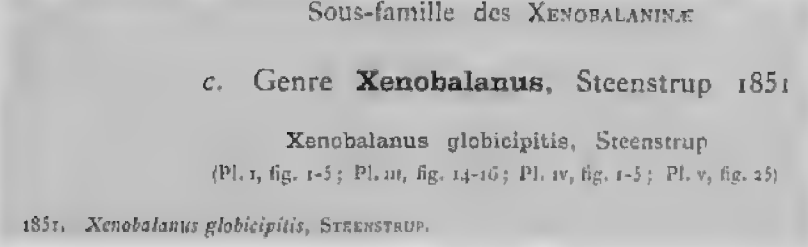| **1897** (scientific journal publication)	**1936** (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
No further text [SEE BELOW FOR PLATE from 1897]	Page # 42 1896 Stn. 638, 27 mai, 43° 26' 20" N., 7° 18' E. Au large de Monaco. Trois Orques sont piqués dans la matinée : une grande femelle de 5ᵐ90, une femelle jeune de 4ᵐ10 et un autre, sans doute un mâle, qui n'a pu être obtenu bien que blessé. Pendant tout le temps que le jeune était pris la mère n'a cessé de tourner autour de lui, à toucher les embarcations, malgré l'hélice	**1936** reprint ["*Station 638, 27 May 1896. 43°26'20"N., 7°18'E. Off the coast of Monaco*"] Also gives this additional text (underlined): ["*Three killer whales are hit in the morning, a longer female of 5m 90, a young female of 4m10 and another, without doubt a male, which could not be obtained although injured. During all the time that the young was caught, the mother kept circling around it, touching the boats, despite the propeller.*"]
No further text [SEE BELOW FOR PLATE from 1897]	Page # 43 Des *Xenobalanus* ont été pris sur la queue et sur la pectorale de la grande femelle. Voir les mesures dans les colonnes 7 et 8 du tableau I. Conservé dans le sel l'appareil génital de la grande femelle et un rein.	**1936** reprint Also gives this additional text (underlined): ["***Xenobalanus*** *were taken from the tail and pectoral of the large female.* *See the measurements in columns 7 and 8 of Table I* [orca measurements]*. The genital tract of the large female and a kidney are preserved in salt.*"]
No further text [SEE BELOW FOR PLATE from 1897]	Page # 48 1902 Stn. 1267, 22 juillet. — 36° 20' N., 5° 09' 45" W. Près de Gibraltar. Un Orque femelle de 4ᵐ70 pesant 1200 kilog. *Xenobalanus* aux deux pectorales et à la caudale. Ventre de l'Orque blanc. Contenu de l'estomac uniquement formé de débris de gros poissons (thon ou espadon ?, os gardés et gros fragments de chair de 1 à 2 kilog.). Cet Orque a été harponné successivement par le Prince et par Wedderburn qui l'a frappé dans le crâne. (Pl. ııı, fig. 8).	**1936** An additional record is noted from 1902 (see **Gruvel 1902** & **(1920)**, below) "*Stn. 1267, 22 july* **[1902]**. *– 36° 20' N., 5° 09' 45" W. Near Gibraltar.* **One female orca of 4m 70 weighing 1,200 kg.** *Xenobalanus* *on both pectorals and on the caudal* [posterior]*. The belly of the orca is white. Contents of the stomach were only composed of big fish debris. (tuna and swordfish? untouched bones and big fragments of meat from 1 to 2 kilos) This orca was harpooned successively by the Prince and by Wedderburn who hit it in the skull. (Pl, III, fig 8).*"

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
No further text [SEE BELOW FOR PLATE from 1897]	Page # 64, Légende de la Planche **II** [*"Legend plate II"*] LÉGENDE DE LA PLANCHE II Pages 4-9. ORCA ORCA (Müller) près de Gibraltar. Stn. 1267, 22 juillet 1902. 1 mâle capturé .... 48 Fig. 4. Orque soufflant tout près de la *PRINCESSE ALICE II*. Coll. R. n° 511. — 5. A la poursuite des Orques. Le Prince Albert ramant avec l'équipage de la baleinière. Coll. R. n° 514. — 6. A la poursuite des Orques dont quatre montrent leur nageoire dorsale. Le Prince prêt à tirer. Coll. R. n° 515. — 7-8. A la poursuite des Orques. Le Prince prêt à tirer. Coll. R. n°s 521-522. — 9. Rencontre des deux baleinières du Prince et de Wedderburn après la capture d'un Orque. Coll. R. n° 513.	[See **Gruvel (1920)** below for original details] [*"ORCA ORCA (Müller) near Gibraltar. Stn. [station]* ***1267** **22nd July 1902**, **1 male captured** ...[page].48* *Fig. 4. Orca blowing near the Princess Alice II. Coll. R. n° 511* *-5. Going after the orca. The Prince Albert is rowing with the crew of the whaler. Coll. R. no 514* *-6. Going after the orca from which 4 are showing their dorsal fin. The Prince is ready to shoot. Coll.R.no 515* *-7-8 Going after the* [**male**] *orca. The Prince is ready to shoot. Coll. R. n°s 521-522.* *-9. Meeting of two whalers of the Prince and of Wedderburn after the capture of one orca.Coll.R.no 513"*] [Note: Fig. 5 (not reproduced here) only pictured the men rowing, no orca visible.]
No further text [SEE BELOW FOR PLATE from 1897]	Page # 66, Légende de la Planche **III** [*"Legend plate III"*] LÉGENDE DE LA PLANCHE III (suite) Pages Fig. 8. ORCA orca Müller ...... 48 Orque pendu, montrant bien tout le système de coloration de la face ventrale. Photographie du professeur Portier. Stn. 1267. (Voir aussi Pl. II, fig. 4-9).	**1936** **[19020722]** [*"Fig. 8. ORCA ORCA Müller .... [page] 48* *Orca hanging, showing the coloration system on the ventral side. Photograph taken by Professor Portier.* ***Stn. 1267** (See also PL. II, fig. 4-9)"*]
No further text [SEE BELOW FOR PLATE from 1897]	Page # 67 Légende de la Planche **IV** [*"Legend plate IV"*] LÉGENDE DE LA PLANCHE IV — 9-10. ORCA orca Müller ...... 42 Capture au large de Monaco. Stn. 638, 27 mai 1896. On voit fig. 10 (Coll. R. Ha. 44.9) l'animal remorqué par la *PRINCESSE ALICE I* vers Monaco où la fig. 9 (Coll. R. Ha. 43.9) le montre échoué au fond du port. Debout le baleinier Wedderburn et des matelots du yacht. Remarquer la forme de la nageoire pectorale et la tache blanche près de l'œil.	**1936** [*"-9-10. ORCA ORCA Müller .... [page] 42* *Captured off* ***Monaco. Stn 638, 27 May 1896**. We see fig. 10 (Coll. R. Ha. 44.9) the animal was towed by the Princess Alice I towards Monaco where fig. 9 (Coll. R. Ha. 43.9) shows it beached in the port. Standing up* [by the orca is] *the whaler Wedderburn and some sailors of the yacht. Notice the shape of the pectoral fin and the white mark near the eye."*]

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. **II**-(Fig. 4) 	**Richard & Neuville (1936)** Note: Caption is on page 64 (see above) [*Fig. 4. Orca blowing near the Princess Alice II. Coll. R. nº 511*]
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. **II**-(Fig. 6) 	**Richard & Neuville (1936)** Note: Caption is on page 64 (see above) [Fig.] *-6. Going after the orca from which 4 are showing their dorsal fin. The Prince is ready to shoot. Coll.R.no 515*]

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. **II**-(Fig. 7-8) 7 8	**Richard & Neuville (1936)** Note: Caption is on page 64 (see above) [[Fig.]-*7-8 Going after the orca. The Prince is ready to shoot. Coll. R. n°s 521-522.* [Note: Fig. 8 shows an adult male orca (closeup not in the original).]
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. **III** (Fig. 8) 8	**Richard & Neuville (1936)** Note: Caption is on page 64 (see above) ["*Fig. 8. ORCA ORCA Müller .... [page] 48 Orca hanging, showing the coloration system on the ventral side. Photograph taken by Professor Portier. Stn. 1267 (See also Pl. II, fig. 4-9)*"] [page 48; *This orca was harpooned successively by the Prince and by Wedderburn who hit it in the skull. (Pl, III, fig 8)*"] [NOTE: This animal is a female, based on the pigmentation of the urogenital area and the pectoral fins, yet the plate caption list (page 64 and see above) states that a male was captured.]

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. IV Fig. 9 9	**Richard & Neuville (1936)** Note: Caption is on page 67 (see above) ["-9-10. ORCA ORCA *Müller ……. [page] 42* *Captured off **Monaco. Stn 638, 27 May 1896**. We see fig. 10 (Coll. R. Ha. 44.9) the animal was towed by the Princess Alice I towards Monaco where fig. 9 (Coll. R. Ha. 43.9) shows it beached in the port. Standing up [by the orca is] the whaler Wedderburn and some sailors of the yacht. Notice the shape of the pectoral fin and the white mark near the eye.*"]
No further text [SEE BELOW FOR PLATE from 1897]	DOC. CÉTACÉS Pl. IV Fig 10 10	**1936** ["*Fig. 8. ORCA ORCA Müller ……. [page] 42* ["-9-10. ORCA ORCA *Müller ……. [page] 42* *Captured off **Monaco. Stn 638, 27 May 1896**. We see fig. 10 (Coll. R. Ha. 44.9) the animal was towed by the Princess Alice I towards Monaco…*]

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
Page # 108 PL VIII Cetacean catches during Princess Alice campaign **1896**. Fig. 4 (dead female). [NOTE: the orca is the upper panel of four images in PL VIII]	Page # 98/104 DOC. CÉTACÉS Pl. **VII** [identical image to Fig. 4 in Richard & Neuville 1896] [Note: the orca is the upper panel of two images, in Fig. 12.]	**Richard & Neuville (1936)** [Note: There is no Caption for Fig. 12, it is only referred to in the text.] [Figure 12. This is the Orca captured off the Coast of **Monaco**.]
[MANUSCRIPT ENDS]	**[MANUSCRIPT ENDS]**	

Table S-1.2. Records of *Xenobalanus globicipitis*. In 1902 a female orca was captured (see Table S-1.1). We present here how that record has been represented in the early literature, with details from **Gruvel (1902) & (1920)** *verses* **Richard (1936)**.

Gruvel (1920) (chapter) (book)	Gruvel (1920) (chapter) (book) [Translation]	Richard & Neuville (1936) (chapter) [Reprints bound into one volume]
Cover Résultats Campagnes Scientifiques accomplies sur son yacht par Albert 1er Prince Souverain de Monaco Publiés sous sa Direction avec le concours de M. Jules Richard Docteur es-sciences, charge des Travaux zoologiques á bord. Fascicule LIII Cirrhipeds provenant des campagnes scientifique de S. A. S. le Prince de Monaco (1885-1913) par A. Gruvel. Avec Sept Planches. Imprimedie de Monaco. 1920.	**Cover** ["*Results from the Scientific Expeditions of HSH the Prince of Monaco (1885–1913). Results of scientific campaigns compiled on his Yacht by Albert 1st Prince Sovereign Prince of Monaco. Under his direction with the assistance of Mr. Jules Richard Doctor of Sciences, in charge of the Zoological works on board. Fascicle LIII. Cirripeds from the scientific campaigns of H.S.H. the Prince of Monaco (1885-1913) by A. Gruvel. With Seven Plates. Printed by Monaco. 1920.*"]	**Cover** [See details above] ["*On some cetaceans observed during the campaigns of the Princess-Alice yacht*"]
[page 55, Section heading]	[page 55, Section heading] ["*Sub-family of* XENOBALANINÆ *c. Genus **Xenobalanus**, Steenstrup 1851* [2] *Xenobalanus globicipits, Steenstrup*"]	

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[2] Steenstrup described Xenobalanus in 1851, however he didn't publish his description until 1852. Steenstrup J.J.S. 1852. On *Xenobalanus globicipitis*, en ny Cirriped-Slaegt af Coronula familien. Videnskabelige meddelelser fra den Naturhistoriske forening i Kjöbenhavn.62–64. (http://www.marinespecies.org/aphia.php?p=taxdetails&id=106237)

Gruvel (1920) (chapter) (book)	Gruvel (1920) (chapter) (book) [Translation]	Richard & Neuville (1936) (chapter) [Reprints bound into one volume]
page 56 — 56 — Campagne de 1896 : Stn. 638, sur un Orque *(Orca gladiator)*. Campagne de 1902 : Stn. 1267, sur un Orque *(Orca gladiator)*. Orca capture details (no mention of *Xenobalanus* here, however see pages 76-77 below)	**page 56:** [Both records listed under the **Xenobalanus** heading (see page 55)] "Campagne de **1896 Stn. 638** sur un orque (Orca gladiator)" [*"Expedition of 1896 Station. 638 on an orca (Orca gladiator)"*] "1902 - Stn 1267 sur un orque (Orca gladiator)" [*"1902 - Station 1267 on an orca (Orca gladiator)"*] **[ORCA RELEVANT TEXT ENDS]**	**1936** [page 48 (3rd paragraph)] Stn. 1267, 22 juillet. — 36° 20' N., 5° 09' 45" W. Près de Gibraltar. Un Orque femelle de 4m70 pesant 1200 kilog. *Xenobalanus* aux deux pectorales et à la caudale. Ventre de l'Orque blanc. Contenu de l'estomac uniquement formé de débris de gros poissons (thon ou espadon ?, os gardés et gros fragments de chair de 1 à 2 kilog.). Cet Orque a été harponné successivement par le Prince et par Wedderburn qui l'a frappé dans le crâne. (Pl. III, fig. 8). [*Stn. 1267, 22 July* [1902]. – *36° 20' N., 5° 09' 45" W. **Near Gibraltar. One female orca of 4m 70** weighing 1,200 kg. **Xenobalanus** on both pectorals and on the caudal* [posterior]. *The belly of the orca is white. Contents of the stomach were only composed of big fish debris. (tuna and swordfish? untouched bones and big fragments of meat from 1 to 2 kilos) This orca was harpooned successively by the Prince and by Wedderburn who hit it in the skull. (Pl, III, fig 8)"*]

Gruvel (1902) in (1920)

Gruvel (1920) makes reference to two expeditions in which *Xenobalanus* was collected from orca. One was the **18960527** record (**Monaco**).

pages 72-73
[*"**Station Number: 638 | Date: 18960527 | Location: Off Monaco** 43° 26' 20" N. 7°18 E.| Depth in metres: Surface | Harvest method: Harpoon | Species collected: **Xenobalanus globicipitis** Steenst. (sur Orca gladiator, caudal).*"]

NUMÉRO de STATION	DATE	LOCALITÉ LATITUDE	LONGITUDE (Greenwich)	PROFONDEUR en MÈTRES	NATURE DU FOND	PROCÉDÉ de RÉCOLTE	ESPÈCES RECUEILLIES
			CAMPAGNE DE 1896				
638	27 mai	43° 26' 20" N. Au large de Monaco	7° 18' W.	Surface		Harpon	*Xenobalanus globicipitis* Steenst. (sur *Orca gladiator*, caudale).
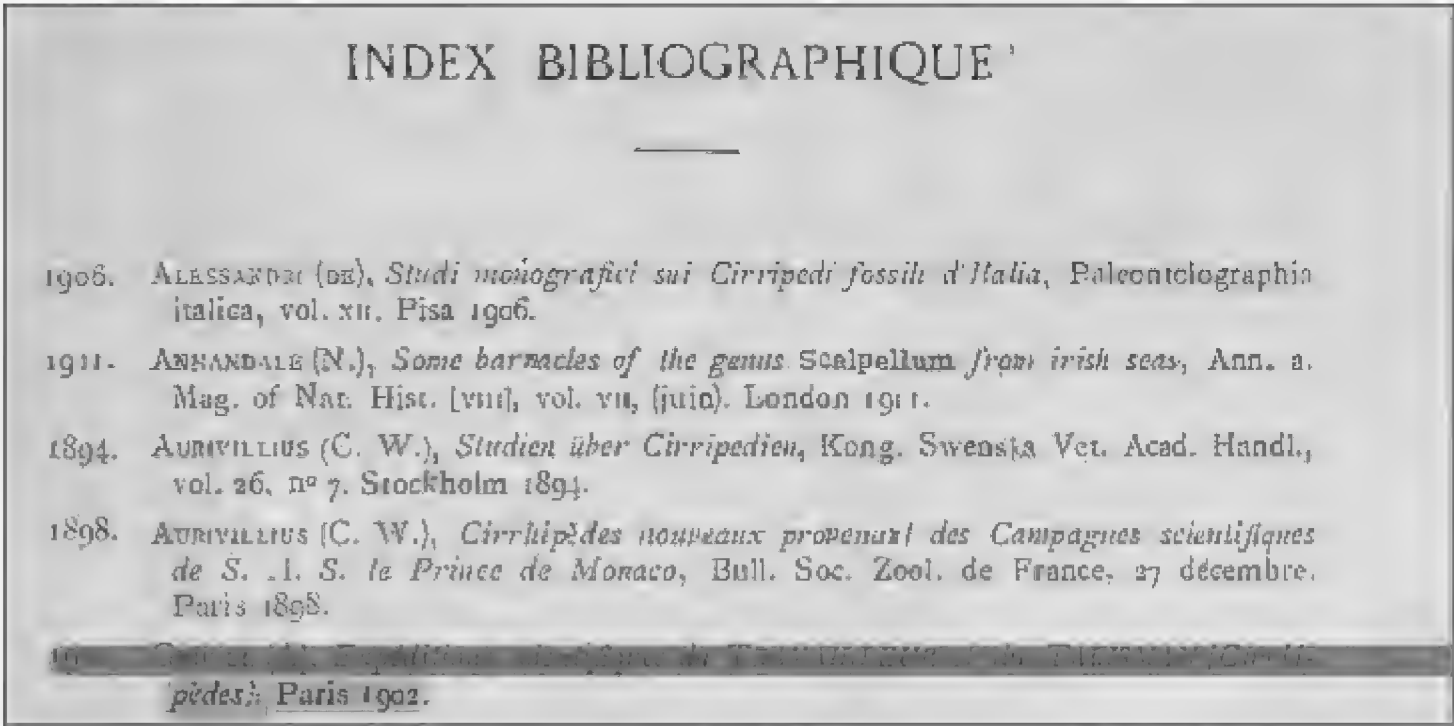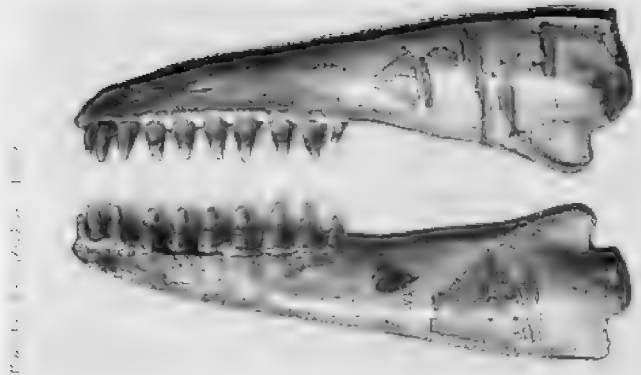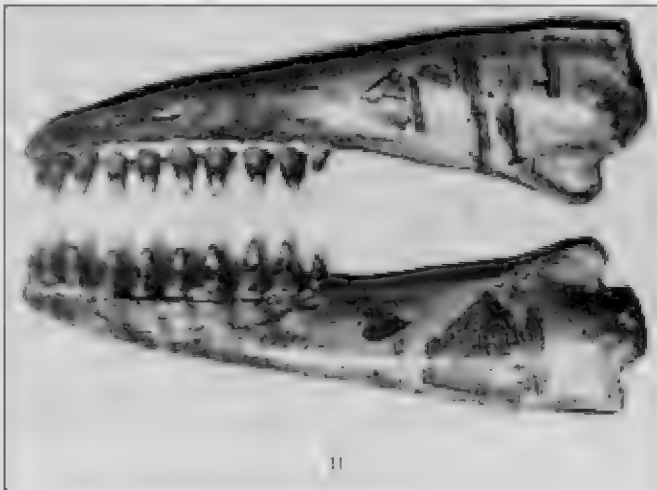
Gruvel (1920). An orca was captured on **19020722**. This is the same date as the 'near Gibraltar' record in **Richard & Neuville (1936),** which was actually captured off the coast of **La Chullera, Spain**

pages 76-77 [*"**Station Number: 1267** | **Date: 19020722** | **Location: 36° 20' N. 5°09' 45" W.** | Depth in metres: Surface | Harvest method: Harpoon | Species collected; **Xenobalanus globicipitis** Steenst. (sur Orca gladiator)."*]

NUMÉRO de STATION	DATE	LOCALITÉ		PROFONDEUR en MÈTRES	NATURE DU FOND	PROCÉDÉ de RÉCOLTE	ESPÈCES RECUEILLIES
		LATITUDE	LONGITUDE (Greenwich)				
		CAMPAGNE				DE 1902	
1267	22 juillet	36 20' N.	5 09' 45" W.	Surface		Harpon	Xenobalanus globicipitis Steenst. (sur Orca gladiator).

The only 1902 citation in Gruvel (1920) is **Gruvel J.A. (1902)**. Chapter 1, Expéditions scientifiques du "Travailleur" et du "Talisman" : Cirrhipèdes. Paris 1902.

This was a chapter in the book:
In: A M-E & Perrier E, editors. Expéditions scientifiques du "Travailleur" et du "Talisman" Pendant les années 1880, 1881, 1882, 1883. Masson et Cie, Éditeurs, Libraires de L'Académie de Médecine. Paris, 1-178 + VII Plates.

NOTE: there is no mention of any orca captures and the only mention of *Xenobalanus* is a generalised description of the barnacle and that it is *"found on whales"* [transaltaion]. Therefore, the Gruvel (1920) publication appears to be the first documentation of the 19020722 La Chullera, Spain record.

page 87 Index Bibliography
1902 reference highlighted.
It is possible that the 1902 record of *Xenobalanus globicipitis* on *Orcinus gladiator* is contained within this reference

INDEX BIBLIOGRAPHIQUE

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1911. ANNANDALE (N.), Some barnacles of the genus Scalpellum from irish seas, Ann. a. Mag. of Nat. Hist. [VIII], vol. VII, (juin). London 1911.

1894. AURIVILLIUS (C. W.), Studien über Cirripedien, Kong. Swenska Vet. Acad. Handl., vol. 26, nº 7. Stockholm 1894.

1898. AURIVILLIUS (C. W.), Cirrhipèdes nouveaux provenant des Campagnes scientifiques de S. A. S. le Prince de Monaco, Bull. Soc. Zool. de France, 27 décembre. Paris 1898.

. . . . pèdes.: Paris 1902.

Table S-1.3 For clarity and to ensure that this record is not conflated with the two previous records already discussed in Tables S-1.1, S-1.2. Two mandibles from an orca (purchased from the Azores) are illustrated in both **Richard & Neuville (1897) and Richard & Neuville (1936)**. *Xenobalanus globicipitis* is not associated with this record.

1897 (scientific journal publication)	1936 (chapter) [Reprints bound into one volume]	Comparisons, Notes (+ translations)
Page # 108 Pl. VIII Cetacean catches during Princess Alice campaign **1896**. (Fig 1).Note: No caption with the plate, just reference to it in the text.	Page # 98/104 DOC. CÉTACÉS Pl.VII. Figure 11.	These orca mandibles are from the **Azores** and not associated with the captures in Monaco or Spain. **Photographs are identical.** 1897 1936
Page 107-108	Page 16	**Text is identical** (except Plate & Fig. numbers) **1897** & **1936** "*Finally, let us mention an orca lower jaw measuring 0m50 in length so consequently belonging to a still young individual of approximately 3m long. There are ten teeth on each side. This piece was purchased in 1895 by one of us from a whaling ship of Gapellas (São Miguel, Azores). We present a photograph (Pl. VII, Fig. 11). On the outer side of the right jaw we see, engraved, hunting of the sperm whale. The boat shown on the narrower section is a schooner, below is a whaling boat having harpooned a Cachalot [sperm whale]. This one is drawn in a perfectly recognizable way and this drawing is better, in general, than many of those that have been published in special works. In front of the whaling boat we see one other under sail. Below, we see the head of a whale blowing, and the body more emerged from another. Finally, the left side of the jaw represents a three-masted ship and above it a heart and the letters N. R., which are undoubtedly the initials of the whaling artist who made these interesting prints.*" [translation]

Supplemental Material S-2. Details and counts of *Xenobalanus* for each appendage of orca NZOP-005. Details and counts of *Xenobalanus globicipitis* (hereafter referred to as *Xenobalanus*). Images were post-process with TopazLab Stabilise AI and Gigapixel AI[1] and assessed at >200% magnification on a high-definition screen. Although the resulting photographs may have lost some finer details, the overall results allowed for more accurate counts than would be possible with the RAW files from the camera alone.

Figure S-2.1 Dorsal view of both tail flukes of NZOP-005 showing *Xenobalanus* clusters (three or more very close together, labelled as R1-R3 and L1-L2). Singles or pairs are labelled separately. Sub-totals are given on each fluke in white text. See Fig. S-2.2-S-2.5 for details.

	RIGHT	LEFT
FLUKE CLUSTERS	R-1, *n*=6	L1, *n*=3
	R-2, *n*=7	L2, *n*=3
	R-3, *n*=4	-
FLUKE SINGLES & PAIRS	*n*=9	*n*=8
Totals	*n*= >26	*n*= >14

Table S-2. 1. Minimum numbers of *Xenobalanus* on tail flukes of NZOP-005.

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[1] https://topazlabs.com/
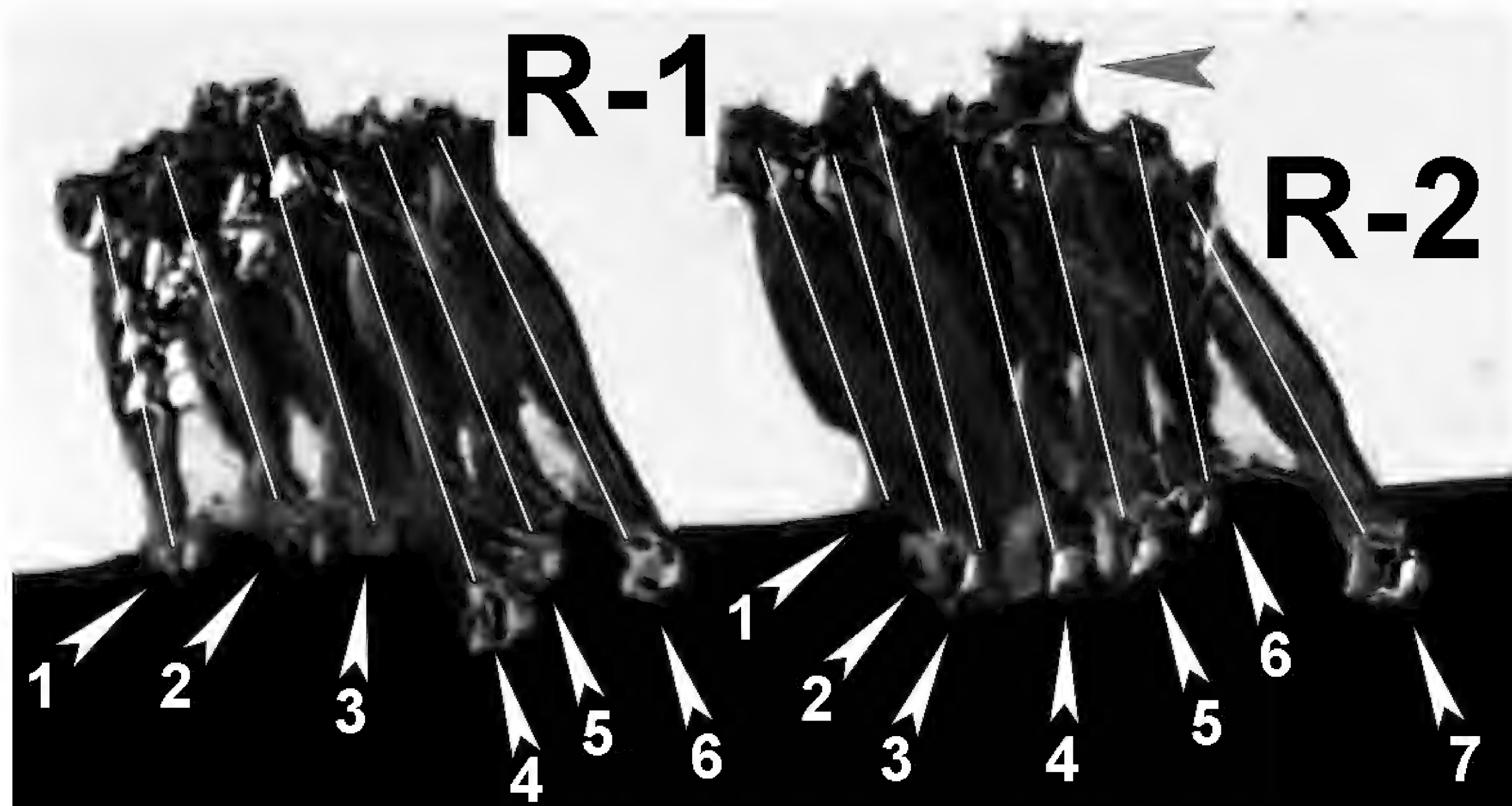
Figure S-2.2 Close-up of *Xenobalanus* clusters R-1 & R-2 (see Fig. S-2.1 for placement on right fluke). Labelling was done by assessing basal plates (typically indicated by a pale area, or a rounded area of similar size to its conspecifics) and drawing a line between the base and the head of the barnacle (white lines). R-1 has at least six barnacles and R2 has at least seven. The yellow arrow indicates a possible 8th *Xenobalanus* in cluster R-2, which was not included in the count in Table S-2.1.
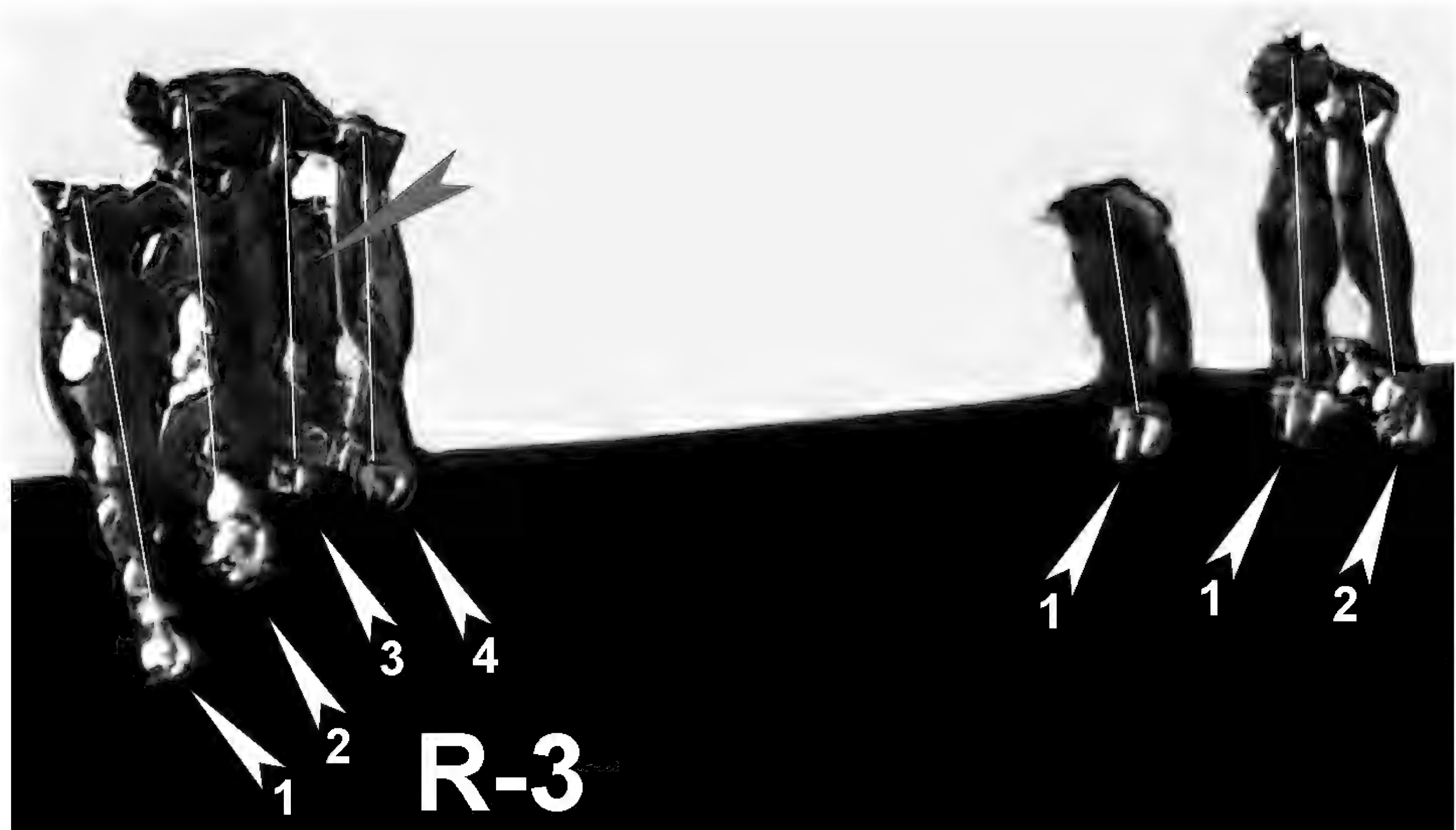
Figure S-2.3. Close-up of R-3 with four barnacles. To the right of R-3 are three barnacles (a single and a pair) which are more widely spaced (see Fig. S-2.1 for placement on right fluke). The yellow arrow indicates a possible 4th *Xenobalanus* in cluster R-3. This was not included in the count in Table S-21.
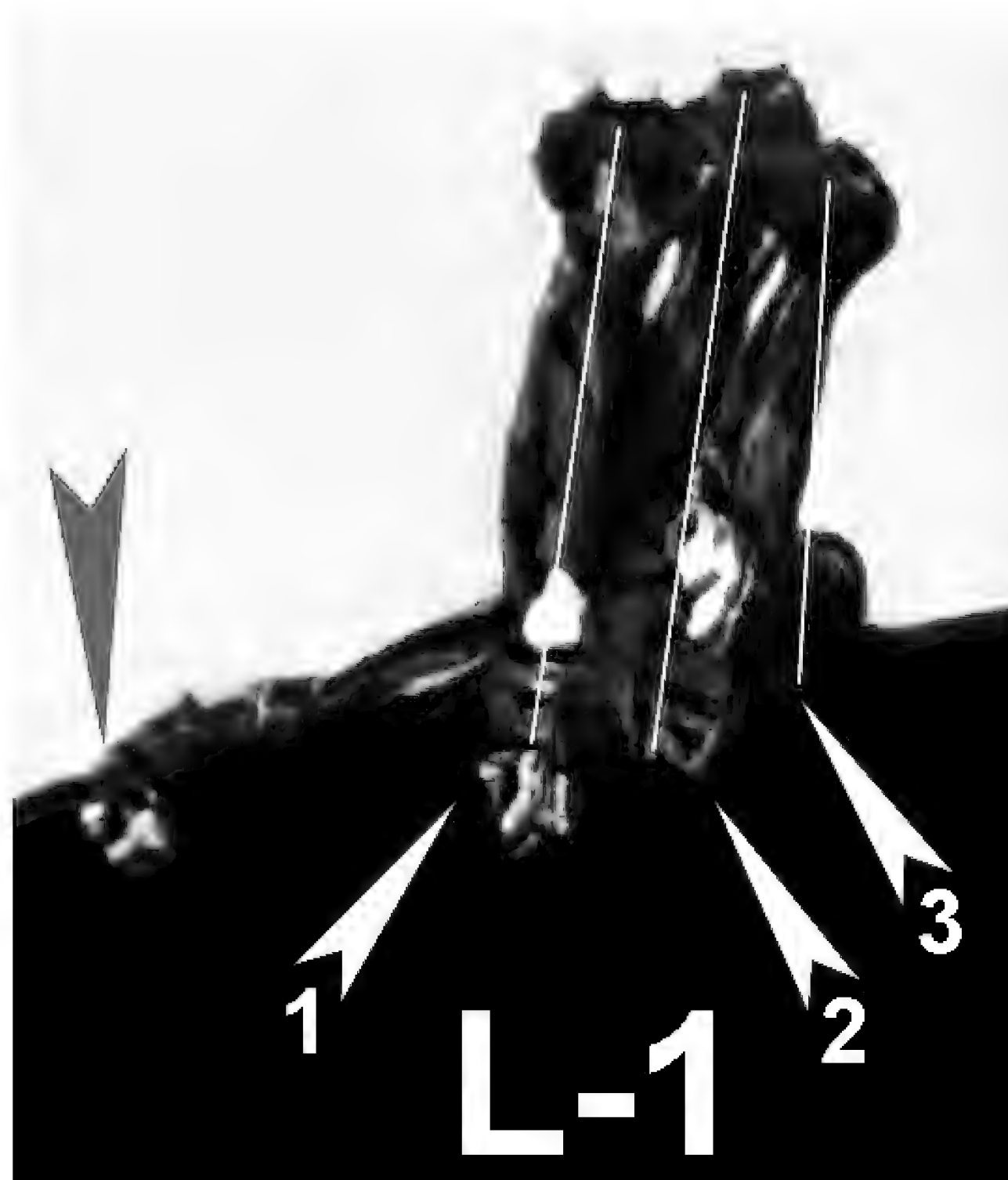
Figure S-2.4. Close-up of L-1 (see Fig. S-2.1 for placement on left fluke). The yellow arrow indicates a possible 4th *Xenobalanus*, which appears to be lying flat and in behind L-1, which was not included in the count in Table S-2.1.
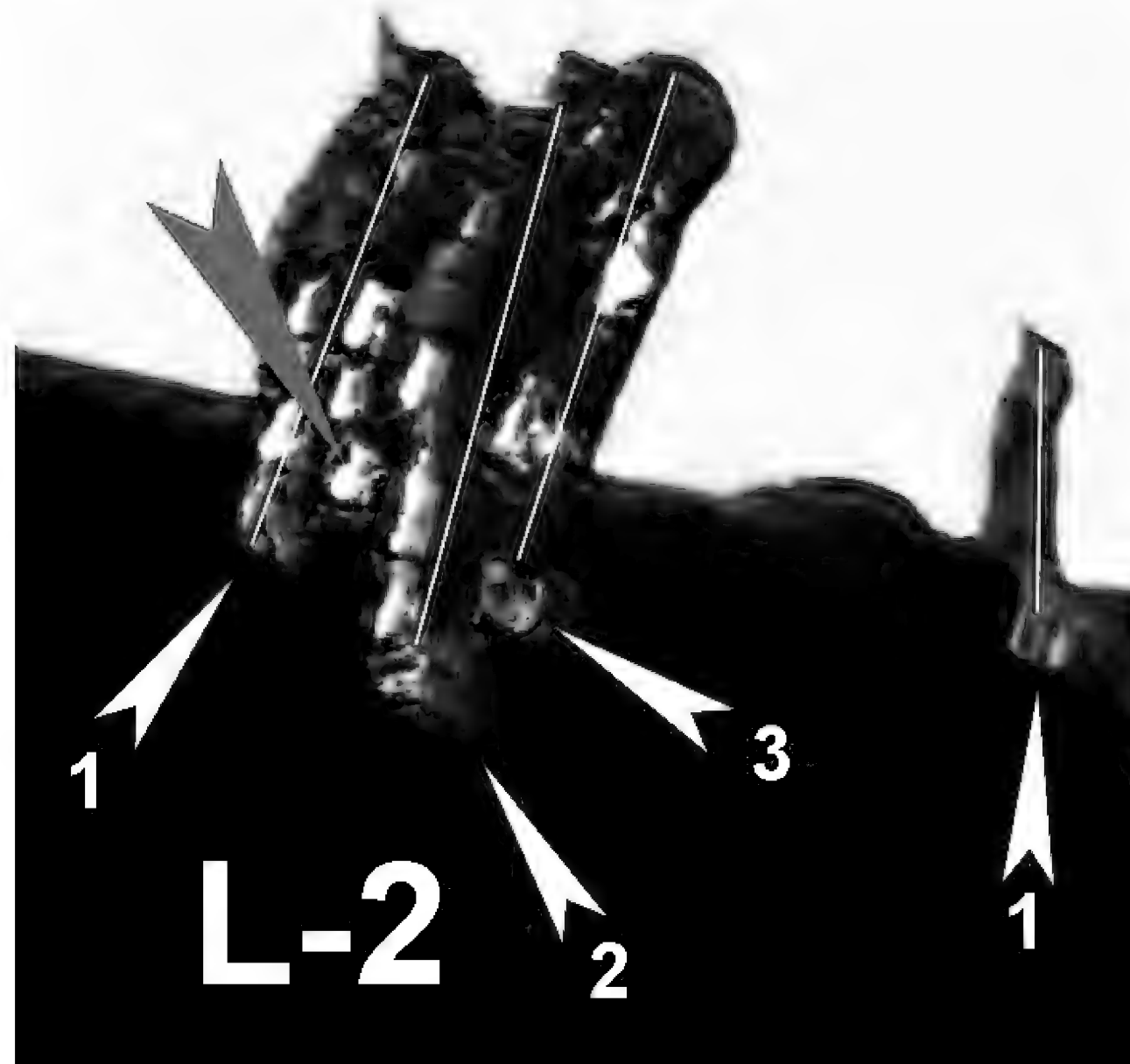
Figure S-2.5. Close-up of L-2 (see Fig. S-2.1 for placement on left fluke). Yellow arrow indicates a possible 4th *Xenobalanus* in L-2, which was not included in the count in Table S-2.1.
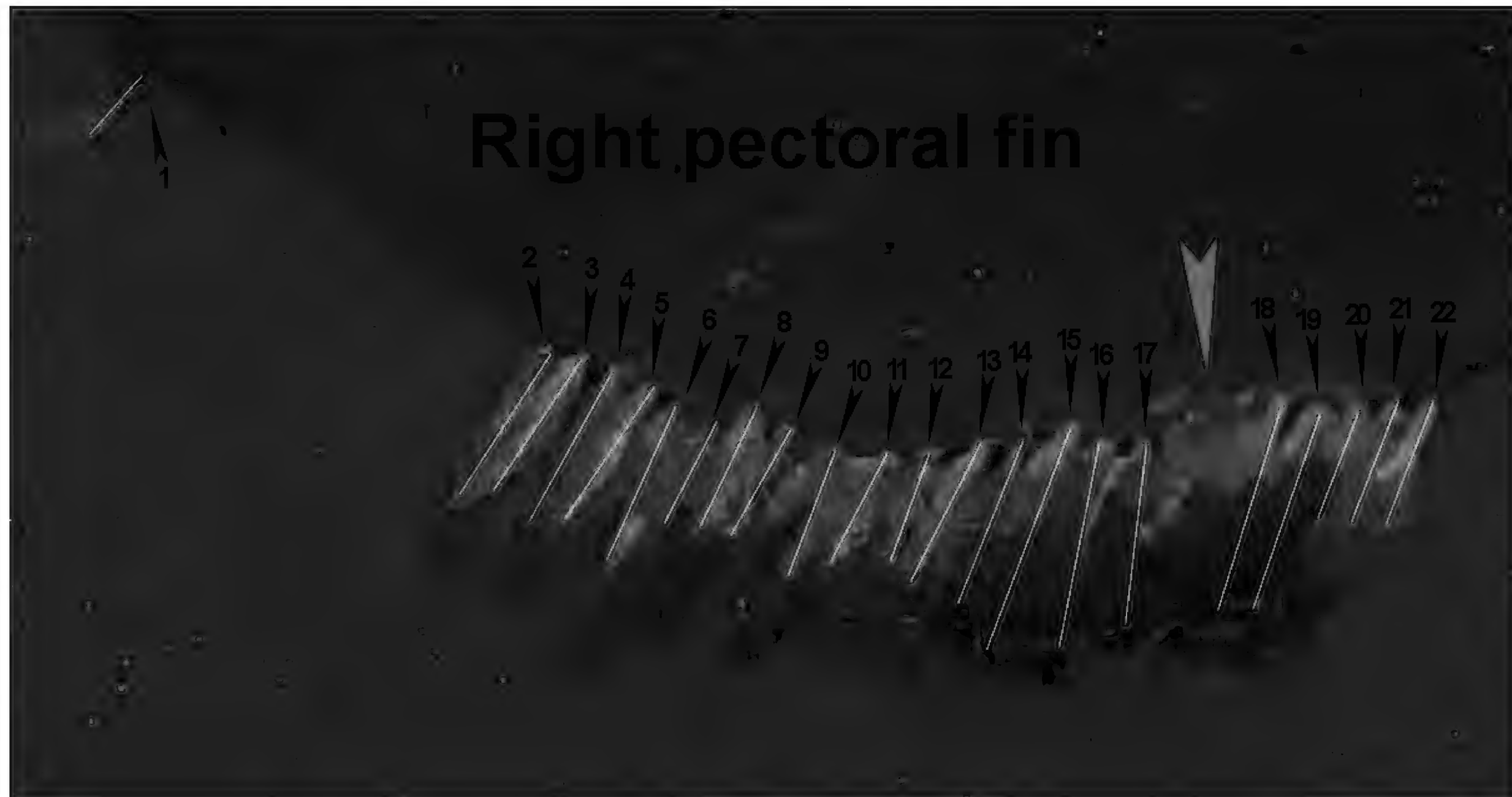
Figure S-2.6. Close-up of right pectoral fin, showing >22 *Xenobalanus*. One barnacle (#1) is at the left of the frame. The yellow arrow indicates an area where it was not possible to count how many barnacles were present but based on the spacing of the barnacles on either side, there may be three or more. These were not included in the count in Table S-2.2.
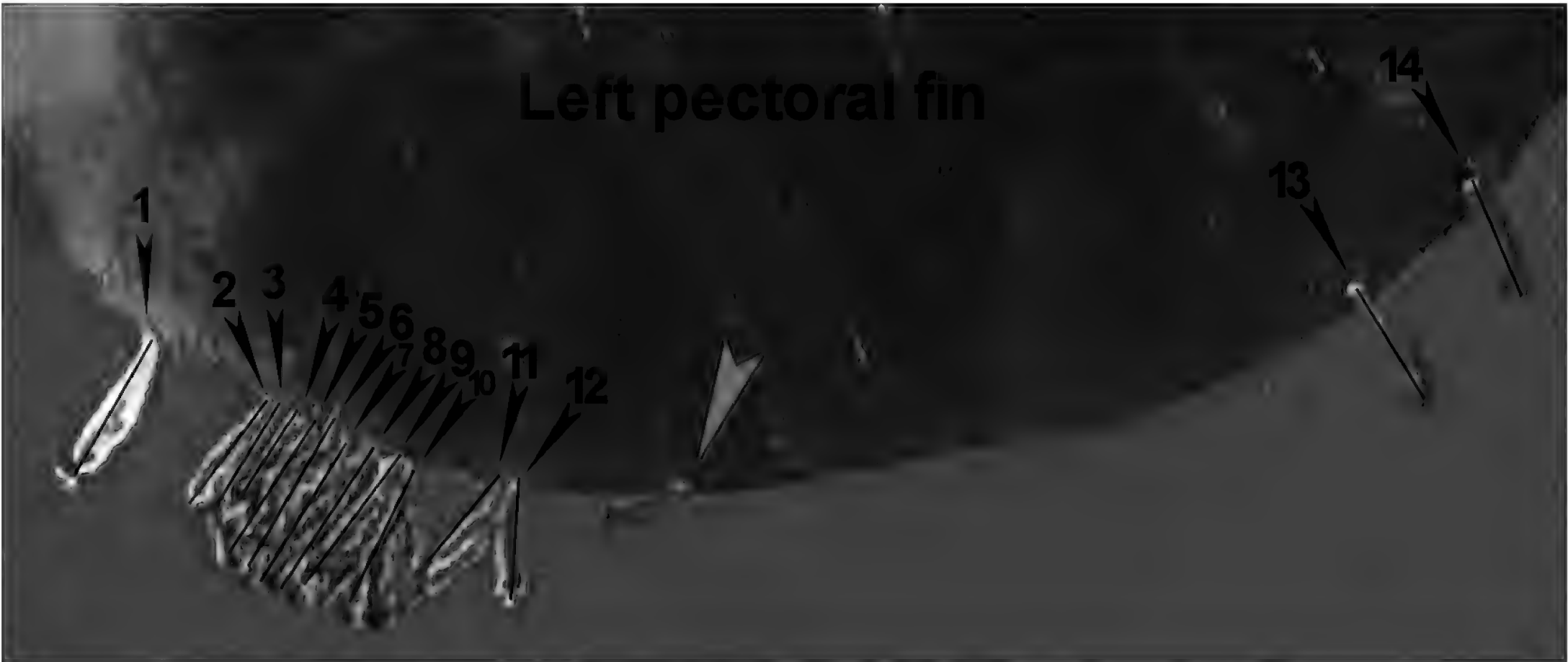
Figure S-2.7. Close-up of left pectoral fin. The yellow arrow indicates a possible 15th *Xenobalanus,* which was not included in the count in Table S-2.2.

	RIGHT	LEFT	APPENDAGE TOTAL
FLUKE	*n= >26*	*n= >14*	*n= >40*
PECTORAL	*n= >22*	*n= >14*	*n= >36*
subtotals	*n=48*	*n= 28*	-
DORSAL FIN	*n=3*		*n=3*
TOTAL	**_n= >79_**		

Table S-2.2. Numbers of *Xenobalanus* on appendages of NZOP-005, with higher numbers on her right side. The total number of >79 is conservative, given that clusters can obscure individuals and movement of appendages can result in some barnacles not being visible at all times.

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